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(54) Title: PLANT FARNESYLTRANSFERASES

(57) Abstract

This invention relates to an isolated nucleic acid fragment encoding a farnesyltransferase subunit. The invention also relates to the construction of a chimeric gene encoding all or a portion of the farnesyltransferase subunit, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of the farnesyltransferase subunit in a transformed host cell.

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TITLE
PLANT FARNESYLTRANSFERASES

FIELD OF THE INVENTION

This invention is in the field of plant molecular biology. More specifically, this invention pertains to nucleic acid fragments encoding farnesyltransferase subunits in plants and seeds.

BACKGROUND OF THE INVENTION

Lipids and proteins associate covalently to form lipid-linked proteins and noncovalently to form lipoproteins. The lipid portions of lipid-linked proteins anchor their attached proteins to membranes and mediate protein-protein interactions. Proteins form covalent attachments to lipids in several ways, one of which is the covalent attachment of isoprenoid groups, mainly the C_{15} farnesyl and C_{20} geranylgeranyl residues.

In mammals, geranylgeranyltransferase is known to catalyze the transfer of a geranyl-geranyl moiety from geranylgeranyl pyrophsophate to both cysteines in Rab proteins (Farnsworth, C. C. et al. (1994) *Proc Natl Acad Sci U S A 91(25)*:11963-11967). Rab proteins are Ras-related small GTPases that are geranylgeranylated on cysteine residues located at or near their C termini. Farnesyltransferase catalyzes the addition of farnesyl groups to the C termini of protein such as Ras.

Mammalian protein geranylgeranyl transferases types 1 and 2 are heterodimers composed of an alpha and beta subunit. The alpha subunit shows homology to the alpha subunits of a closely related enzyme, farnesyltransferase. Farnesyltransferases have been described in pea, tomato, and *Arabidopsis*, but have not been described in monocots (Yang et al. (1993) *Plant Physiology 101*:667-674). Plant farnesyltransferases also consist of alpha and beta subunits. The beta subunit is responsible for peptide-binding and contains a catalytic zinc ion. The beta subunit belongs to the protein prenyltransferase beta subunit family. The geranylgeranyl transferase beta subunit also belongs to the protein prenyltransferase beta subunit family. The beta subunits of the type 1 and 2 geranylgeranyltransferases have not been previously described in plants. Work done in yeast has established that protein geranylgeranyltransferases are distinct from the closely related protein farnesyltransferases.

It has been shown that defects in farnesyltransferase activity enhances plant hormone abscisic acid (ABA) levels. In a normal plant ABA levels increase in response to water deficits. An increase ABA in leaf tissue triggers the closure of leaf stomata to decrease water loss via transpiration (Pei et al. (1998) *Science 282*:287-290). Plants with a decrease in farnesyltransferase activity could confer enhanced tolerance to drought stress in plants. Thus, there is a great deal of interest in identifying the genes that encode farnesyltransferase

in plants. These genes may be used in plant cells to control cell growth and produce plants with improved water stress tolerance. Accordingly, the availability of nucleic acid sequences encoding all or a portion of farnesyltransferase proteins would facilitate studies to better understand cell growth in plants, provide genetic tools to control cell growth and improve tolerance to drought in mature plants.

SUMMARY OF THE INVENTION

The present invention relates to isolated polynucleotides comprising a nucleotide sequence encoding a first polypeptide of at least 300 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of a corn farnesyltransferase polypeptide of SEQ ID NO:2, a rice farnesyltransferase polypeptide of SEQ ID NO:4, a soybean farnesyltransferase polypeptide of SEQ ID NO:6, a soybean farnesyltransferase polypeptide of SEQ ID NO:8, a wheat farnesyltransferase polypeptide of SEQ ID NO:10, a corn farnesyltransferase polypeptide of SEQ ID NO:12, a rice farnesyltransferase polypeptide of SEQ ID NO:14, a soybean farnesyltransferase polypeptide of SEQ ID NO:16, and a soybean farnesyltransferase polypeptide of SEQ ID NO:18. The present invention also relates to an isolated polynucleotide comprising the complement of the nucleotide sequences described above. It is preferred that the isolated polynucleotides of the claimed invention consists of regions of the isolated polynucleotide selected from the group consisting of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, and 17 that codes for the polypeptide selected from the group consisting of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16 and 18. The present invention also relates to an isolated polynucleotide comprising a nucleotide sequences of at least one of 40 (preferably 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17 and the complement of such nucleotide sequences.

The present invention relates to a chimeric gene comprising an isolated polynucleotide of the present invention operably linked to suitable regulatory sequences.

The present invention relates to an isolated host cell comprising a chimeric gene of the present invention or an isolated polynucleotide of the present invention. The host cell may be eucaryotic, such as a yeast or a plant cell, or procaryotic, such as a bacterial cell. The present invention also relates to a virus, preferably a baculovirus, comprising an isolated polynucleotide of the present invention or a chimeric gene of the present invention.

The present invention relates to a process for producing an isolated host cell comprising a chimeric gene or isolated polynucleotide of the present invention, the process comprising either transforming or transfecting an isolated compatible host cell with a chimeric gene or isolated polynucleotide of the present invention.

The present invention relates to a farnesyltransferase polypeptide of at least 300 amino acids that has at least 80% homology based on the Clustal method of alignment

compared to a polypeptide selected from the group consisting of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18.

The present invention relates to a method of selecting an isolated polynucleotide that affects the level of expression of a farnesyltransferase polypeptide in a plant cell, the method comprising the steps of:

constructing an isolated polynucleotide or chimeric gene of the present invention;

introducing the isolated polynucleotide into a plant cell;

measuring the level of farnesyltransferase polypeptide in the plant cell containing the polynucleotide; and

comparing the level of farnesyltransferase polypeptide in the plant cell containing the isolated polynucleotide with the level of farnesyltransferase polypeptide in a plant cell that does not contain the isolated polynucleotide.

The present invention relates to a method of obtaining a nucleic acid fragment encoding a substantial portion of a farnesyltransferase gene, preferably a plant farnesyltransferase gene, comprising the steps of : synthesizing an oligonucleotide primer comprising a nucleotide sequence of at least one of 40 (preferably 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17 and the complement of such nucleotide sequences; and amplifying a nucleic acid fragment (preferably a cDNA inserted in a cloning vector) using the oligonucleotide primer. The amplified nucleic acid fragment preferably will encode a portion of a farnesyltransferase polypeptide amino acid sequence.

The present invention also relates to a method of obtaining a nucleic acid fragment encoding all or a subsantial portion of the amino acid sequence encoding a farnesyl-transferase protein comprising the steps of: probing a cDNA or genomic library with an isolated polynucleotide of the present invention; identifying a DNA clone that hybridizes with an isolated polynucleotide of the present invention; isolating the identified DNA clone; and sequencing the cDNA or genomic fragment that comprises the isolated DNA clone.

BRIEF DESCRIPTION OF THE SEQUENCE DESCRIPTIONS

The invention can be more fully understood from the following detailed description and the accompanying Sequence Listing which form a part of this application.

Table 1 lists the polypeptides that are described herein, the designation of the cDNA clones that comprise the nucleic acid fragments encoding polypeptides representing all or a substantial portion of these polypeptides, and the corresponding identifier (SEQ ID NO:) as used in the attached Sequence Listing. The sequence descriptions and Sequence Listing attached hereto comply with the rules governing nucleotide and/or amino acid sequence disclosures in patent applications as set forth in 37 C.F.R. §1.821-1.825.

TABLE 1

Farnesyltransferase Subunits

Protein	Clone Designation	SEQ ID NO: (Nucleotide)	(Amino Acid)
Farnesyltransferase Alpha Subunit	cen3n.pk0052.a2	1	2
Farnesyltransferase Alpha Subunit	rls6.pk0017.g2	3	4
Farnesyltransferase Alpha Subunit	src1c.pk002.f15	5	6
Farnesyltransferase Alpha Subunit	sgs2c.pk006.n4	7	8
Farnesyltransferase Alpha Subunit	Contig composed of: wdk2c.pk013.d24 wr1.pk0110.b1	9	10
Farnesyltransferase Beta Subunit	p0127.cntbu.18r	11	12
Farnesyltransferase Beta Subunit	rlr24.pk0007.d6	13	14
Farnesyltransferase Beta Subunit	sfl1.pk0086.h10	15	16
Farnesyltransferase Beta Subunit	sgs2c.pk002.g2	17	18

The Sequence Listing contains the one letter code for nucleotide sequence characters and the three letter codes for amino acids as defined in conformity with the IUPAC-IUBMB standards described in *Nucleic Acids Res. 13*:3021-3030 (1985) and in the *Biochemical J. 219 (No. 2)*:345-373 (1984) which are herein incorporated by reference. The symbols and format used for nucleotide and amino acid sequence data comply with the rules set forth in 37 C.F.R. §1.822.

DETAILED DESCRIPTION OF THE INVENTION

In the context of this disclosure, a number of terms shall be utilized. As used herein, a "polynucleotide" is a nucleotide sequence such as a nucleic acid fragment. A polynucleotide may be a polymer of RNA or DNA that is single- or double-stranded, that optionally contains synthetic, non-natural or altered nucleotide bases. A polynucleotide in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA, or synthetic DNA.

As used herein, "contig" refers to a nucleotide sequence that is assembled from two or more constituent nucleotide sequences that share common or overlapping regions of sequence homology. For example, the nucleotide sequences of two or more nucleic acid fragments can be compared and aligned in order to identify common or overlapping

sequences. Where common or overlapping sequences exist between two or more nucleic acid fragments, the sequences (and thus their corresponding nucleic acid fragments) can be assembled into a single contiguous nucleotide sequence.

As used herein, "substantially similar" refers to nucleic acid fragments wherein changes in one or more nucleotide bases results in substitution of one or more amino acids, but do not affect the functional properties of the polypeptide encoded by the nucleotide sequence. "Substantially similar" also refers to nucleic acid fragments wherein changes in one or more nucleotide bases does not affect the ability of the nucleic acid fragment to mediate alteration of gene expression by gene silencing through for example antisense or co-suppression technology. "Substantially similar" also refers to modifications of the nucleic acid fragments of the instant invention such as deletion or insertion of one or more nucleotides that do not substantially affect the functional properties of the resulting transcript vis-à-vis the ability to mediate gene silencing or alteration of the functional properties of the resulting protein molecule. It is therefore understood that the invention encompasses more than the specific exemplary nucleotide or amino acid sequences and includes functional equivalents thereof.

For example, it is well known in the art that antisense suppression and co-suppression of gene expression may be accomplished using nucleic acid fragments representing less than the entire coding region of a gene, and by nucleic acid fragments that do not share 100% sequence identity with the gene to be suppressed. Moreover, alterations in a nucleic acid fragment which result in the production of a chemically equivalent amino acid at a given site, but do not effect the functional properties of the encoded polypeptide, are well known in the art. Thus, a codon for the amino acid alanine, a hydrophobic amino acid, may be substituted by a codon encoding another less hydrophobic residue, such as glycine, or a more hydrophobic residue, such as valine, leucine, or isoleucine. Similarly, changes which result in substitution of one negatively charged residue for another, such as aspartic acid for glutamic acid, or one positively charged residue for another, such as lysine for arginine, can also be expected to produce a functionally equivalent product. Nucleotide changes which result in alteration of the N-terminal and C-terminal portions of the polypeptide molecule would also not be expected to alter the activity of the polypeptide. Each of the proposed modifications is well within the routine skill in the art, as is determination of retention of biological activity of the encoded products. Consequently, a polynucleotide comprising a nucleotide sequence of at least one of 40 (preferably 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17 and the complement of such nucleotide sequences may be used in methods of selecting an isolated polynucleotide that affects the expression of a farnesyltransferase polypeptide in a plant cell.

Moreover, substantially similar nucleic acid fragments may also be characterized by their ability to hybridize. Estimates of such homology are provided by either DNA-DNA or DNA-RNA hybridization under conditions of stringency as is well understood by those skilled in the art (Hames and Higgins, Eds. (1985) Nucleic Acid Hybridisation, IRL Press, Oxford, U.K.). Stringency conditions can be adjusted to screen for moderately similar fragments, such as homologous sequences from distantly related organisms, to highly similar fragments, such as genes that duplicate functional enzymes from closely related organisms. Post-hybridization washes determine stringency conditions. One set of preferred conditions uses a series of washes starting with 6X SSC, 0.5% SDS at room temperature for 15 min, then repeated with 2X SSC, 0.5% SDS at 45°C for 30 min, and then repeated twice with 0.2X SSC, 0.5% SDS at 50°C for 30 min. A more preferred set of stringent conditions uses higher temperatures in which the washes are identical to those above except for the temperature of the final two 30 min washes in 0.2X SSC, 0.5% SDS was increased to 60°C. Another preferred set of highly stringent conditions uses two final washes in 0.1X SSC, 0.1% SDS at 65°C.

Substantially similar nucleic acid fragments of the instant invention may also be characterized by the percent identity of the amino acid sequences that they encode to the amino acid sequences disclosed herein, as determined by algorithms commonly employed by those skilled in this art. Suitable nucleic acid fragments (polynucleotides) encode amino acid sequences that are 80% identical to the amino acid sequences reported herein. Preferred nucleic acid fragment encode amino acid sequences that are 85% identical to the amino acid sequences reported herein. Most preferred are nucleic acid fragments that encode amino acid sequences that are 95% identical to the amino acid sequences reported herein. Sequence alignments and percent identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS.* 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5.

A "substantial portion" of an amino acid or nucleotide sequence comprises an amino acid or a nucleotide sequence that is sufficient to afford putative identification of the protein or gene that the amino acid or nucleotide sequence comprises. Amino acid and nucleotide sequences can be evaluated either manually by one skilled in the art, or by using computer-based sequence comparison and identification tools that employ algorithms such as BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol. 215*:403-410; see also www.ncbi.nlm.nih.gov/BLAST/). In general, a sequence of ten or more contiguous amino acids or thirty or more contiguous nucleotides is necessary in order to putatively

identify a polypeptide or nucleic acid sequence as homologous to a known protein or gene. Moreover, with respect to nucleotide sequences, gene-specific oligonucleotide probes comprising 30 or more contiguous nucleotides may be used in sequence-dependent methods of gene identification (e.g., Southern hybridization) and isolation (e.g., *in situ* hybridization of bacterial colonies or bacteriophage plaques). In addition, short oligonucleotides of 12 or more nucleotides may be used as amplification primers in PCR in order to obtain a particular nucleic acid fragment comprising the primers. Accordingly, a "substantial portion" of a nucleotide sequence comprises a nucleotide sequence that will afford specific identification and/or isolation of a nucleic acid fragment comprising the sequence. The instant specification teaches amino acid and nucleotide sequences encoding polypeptides that comprise one or more particular plant proteins. The skilled artisan, having the benefit of the sequences as reported herein, may now use all or a substantial portion of the disclosed sequences for purposes known to those skilled in this art. Accordingly, the instant invention comprises the complete sequences as reported in the accompanying Sequence Listing, as well as substantial portions of those sequences as defined above.

"Codon degeneracy" refers to divergence in the genetic code permitting variation of the nucleotide sequence without effecting the amino acid sequence of an encoded polypeptide. Accordingly, the instant invention relates to any nucleic acid fragment comprising a nucleotide sequence that encodes all or a substantial portion of the amino acid sequences set forth herein. The skilled artisan is well aware of the "codon-bias" exhibited by a specific host cell in usage of nucleotide codons to specify a given amino acid. Therefore, when synthesizing a nucleic acid fragment for improved expression in a host cell, it is desirable to design the nucleic acid fragment such that its frequency of codon usage approaches the frequency of preferred codon usage of the host cell.

"Synthetic nucleic acid fragments" can be assembled from oligonucleotide building blocks that are chemically synthesized using procedures known to those skilled in the art. These building blocks are ligated and annealed to form larger nucleic acid fragments which may then be enzymatically assembled to construct the entire desired nucleic acid fragment. "Chemically synthesized", as related to nucleic acid fragment, means that the component nucleotides were assembled *in vitro*. Manual chemical synthesis of nucleic acid fragments may be accomplished using well established procedures, or automated chemical synthesis can be performed using one of a number of commercially available machines. Accordingly, the nucleic acid fragments can be tailored for optimal gene expression based on optimization of nucleotide sequence to reflect the codon bias of the host cell. The skilled artisan appreciates the likelihood of successful gene expression if codon usage is biased towards those codons favored by the host. Determination of preferred codons can be based on a survey of genes derived from the host cell where sequence information is available.

"Gene" refers to a nucleic acid fragment that expresses a specific protein, including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence. "Native gene" refers to a gene as found in nature with its own regulatory sequences. "Chimeric gene" refers any gene that is not a native gene, comprising regulatory and coding sequences that are not found together in nature. Accordingly, a chimeric gene may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature. "Endogenous gene" refers to a native gene in its natural location in the genome of an organism. A "foreign" gene refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, or chimeric genes. A "transgene" is a gene that has been introduced into the genome by a transformation procedure.

"Coding sequence" refers to a nucleotide sequence that codes for a specific amino acid sequence. "Regulatory sequences" refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters, translation leader sequences, introns, and polyadenylation recognition sequences.

"Promoter" refers to a nucleotide sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence is located 3' to a promoter sequence. The promoter sequence consists of proximal and more distal upstream elements, the latter elements often referred to as enhancers. Accordingly, an "enhancer" is a nucleotide sequence which can stimulate promoter activity and may be an innate element of the promoter or a heterologous element inserted to enhance the level or tissue-specificity of a promoter. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic nucleotide segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental conditions. Promoters which cause a nucleic acid fragment to be expressed in most cell types at most times are commonly referred to as "constitutive promoters". New promoters of various types useful in plant cells are constantly being discovered; numerous examples may be found in the compilation by Okamuro and Goldberg (1989) *Biochemistry of Plants 15*:1-82. It is further recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, nucleic acid fragments of different lengths may have identical promoter activity.

The "translation leader sequence" refers to a nucleotide sequence located between the promoter sequence of a gene and the coding sequence. The translation leader sequence is present in the fully processed mRNA upstream of the translation start sequence. The translation leader sequence may affect processing of the primary transcript to mRNA, mRNA stability or translation efficiency. Examples of translation leader sequences have been described (Turner and Foster (1995) *Mol. Biotechnol. 3*:225-236).

The "3' non-coding sequences" refer to nucleotide sequences located downstream of a coding sequence and include polyadenylation recognition sequences and other sequences encoding regulatory signals capable of affecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor. The use of different 3' non-coding sequences is exemplified by Ingelbrecht et al. (1989) *Plant Cell 1*:671-680.

"RNA transcript" refers to the product resulting from RNA polymerase-catalyzed transcription of a DNA sequence. When the RNA transcript is a perfect complementary copy of the DNA sequence, it is referred to as the primary transcript or it may be a RNA sequence derived from posttranscriptional processing of the primary transcript and is referred to as the mature RNA. "Messenger RNA (mRNA)" refers to the RNA that is without introns and that can be translated into polypeptide by the cell. "cDNA" refers to a double-stranded DNA that is complementary to and derived from mRNA. "Sense" RNA refers to an RNA transcript that includes the mRNA and so can be translated into a polypeptide by the cell. "Antisense RNA" refers to an RNA transcript that is complementary to all or part of a target primary transcript or mRNA and that blocks the expression of a target gene (see U.S. Patent No. 5,107,065, incorporated herein by reference). The complementarity of an antisense RNA may be with any part of the specific nucleotide sequence, i.e., at the 5' non-coding sequence, 3' non-coding sequence, introns, or the coding sequence. "Functional RNA" refers to sense RNA, antisense RNA, ribozyme RNA, or other RNA that may not be translated but yet has an effect on cellular processes.

The term "operably linked" refers to the association of two or more nucleic acid fragments on a single nucleic acid fragment so that the function of one is affected by the other. For example, a promoter is operably linked with a coding sequence when it is capable of affecting the expression of that coding sequence (i.e., that the coding sequence is under the transcriptional control of the promoter). Coding sequences can be operably linked to regulatory sequences in sense or antisense orientation.

The term "expression", as used herein, refers to the transcription and stable accumulation of sense (mRNA) or antisense RNA derived from the nucleic acid fragment of the invention. Expression may also refer to translation of mRNA into a polypeptide. "Antisense inhibition" refers to the production of antisense RNA transcripts capable of suppressing the expression of the target protein. "Overexpression" refers to the production

of a gene product in transgenic organisms that exceeds levels of production in normal or non-transformed organisms. "Co-suppression" refers to the production of sense RNA transcripts capable of suppressing the expression of identical or substantially similar foreign or endogenous genes (U.S. Patent No. 5,231,020, incorporated herein by reference).

"Altered levels" refers to the production of gene product(s) in transgenic organisms in amounts or proportions that differ from that of normal or non-transformed organisms.

"Mature" protein refers to a post-translationally processed polypeptide; i.e., one from which any pre- or propeptides present in the primary translation product have been removed. "Precursor" protein refers to the primary product of translation of mRNA; i.e., with pre- and propeptides still present. Pre- and propeptides may be but are not limited to intracellular localization signals.

A "chloroplast transit peptide" is an amino acid sequence which is translated in conjunction with a protein and directs the protein to the chloroplast or other plastid types present in the cell in which the protein is made. "Chloroplast transit sequence" refers to a nucleotide sequence that encodes a chloroplast transit peptide. A "signal peptide" is an amino acid sequence which is translated in conjunction with a protein and directs the protein to the secretory system (Chrispeels (1991) *Ann. Rev. Plant Phys. Plant Mol. Biol. 42*:21-53). If the protein is to be directed to a vacuole, a vacuolar targeting signal (*supra*) can further be added, or if to the endoplasmic reticulum, an endoplasmic reticulum retention signal (*supra*) may be added. If the protein is to be directed to the nucleus, any signal peptide present should be removed and instead a nuclear localization signal included (Raikhel (1992) *Plant Phys. 100*:1627-1632).

"Transformation" refers to the transfer of a nucleic acid fragment into the genome of a host organism, resulting in genetically stable inheritance. Host organisms containing the transformed nucleic acid fragments are referred to as "transgenic" organisms. Examples of methods of plant transformation include *Agrobacterium*-mediated transformation (De Blaere et al. (1987) *Meth. Enzymol. 143*:277) and particle-accelerated or "gene gun" transformation technology (Klein et al. (1987) *Nature (London) 327*:70-73; U.S. Patent No. 4,945,050, incorporated herein by reference).

Standard recombinant DNA and molecular cloning techniques used herein are well known in the art and are described more fully in Sambrook et al. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, 1989 (hereinafter "Maniatis").

Nucleic acid fragments encoding at least a portion of several farnesyltransferase subunits have been isolated and identified by comparison of random plant cDNA sequences to public databases containing nucleotide and protein sequences using the BLAST algorithms well known to those skilled in the art. The nucleic acid fragments of the instant invention may be used to isolate cDNAs and genes encoding homologous proteins from the

same or other plant species. Isolation of homologous genes using sequence-dependent protocols is well known in the art. Examples of sequence-dependent protocols include, but are not limited to, methods of nucleic acid hybridization, and methods of DNA and RNA amplification as exemplified by various uses of nucleic acid amplification technologies (e.g., polymerase chain reaction, ligase chain reaction).

For example, genes encoding other farnesyltransferase alpha or beta subunits, either as cDNAs or genomic DNAs, could be isolated directly by using all or a portion of the instant nucleic acid fragments as DNA hybridization probes to screen libraries from any desired plant employing methodology well known to those skilled in the art. Specific oligonucleotide probes based upon the instant nucleic acid sequences can be designed and synthesized by methods known in the art (Maniatis). Moreover, the entire sequences can be used directly to synthesize DNA probes by methods known to the skilled artisan such as random primer DNA labeling, nick translation, or end-labeling techniques, or RNA probes using available *in vitro* transcription systems. In addition, specific primers can be designed and used to amplify a part or all of the instant sequences. The resulting amplification products can be labeled directly during amplification reactions or labeled after amplification reactions, and used as probes to isolate full length cDNA or genomic fragments under conditions of appropriate stringency.

In addition, two short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols to amplify longer nucleic acid fragments encoding homologous genes from DNA or RNA. The polymerase chain reaction may also be performed on a library of cloned nucleic acid fragments wherein the sequence of one primer is derived from the instant nucleic acid fragments, and the sequence of the other primer takes advantage of the presence of the polyadenylic acid tracts to the 3' end of the mRNA precursor encoding plant genes. Alternatively, the second primer sequence may be based upon sequences derived from the cloning vector. For example, the skilled artisan can follow the RACE protocol (Frohman et al. (1988) *Proc. Natl. Acad. Sci. USA 85*:8998-9002) to generate cDNAs by using PCR to amplify copies of the region between a single point in the transcript and the 3' or 5' end. Primers oriented in the 3' and 5' directions can be designed from the instant sequences. Using commercially available 3' RACE or 5' RACE systems (BRL), specific 3' or 5' cDNA fragments can be isolated (Ohara et al. (1989) *Proc. Natl. Acad. Sci. USA 86*:5673-5677; Loh et al. (1989) *Science 243*:217-220). Products generated by the 3' and 5' RACE procedures can be combined to generate full-length cDNAs (Frohman and Martin (1989) *Techniques 1*:165). Consequently, a polynucleotide comprising a nucleotide sequence of at least one of 40 (preferably 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17 and the complement of such nucleotide sequences may be used in such

methods to obtain a nucleic acid fragment encoding a substantial portion of an amino acid sequence of a thioredoxin polypeptide.

Availability of the instant nucleotide and deduced amino acid sequences facilitates immunological screening of cDNA expression libraries. Synthetic peptides representing portions of the instant amino acid sequences may be synthesized. These peptides can be used to immunize animals to produce polyclonal or monoclonal antibodies with specificity for peptides or proteins comprising the amino acid sequences. These antibodies can be then be used to screen cDNA expression libraries to isolate full-length cDNA clones of interest (Lerner (1984) *Adv. Immunol. 36*:1-34; Maniatis).

The nucleic acid fragments of the instant invention may be used to create transgenic plants in which the disclosed polypeptides are present at higher or lower levels than normal or in cell types or developmental stages in which they are not normally found. This would have the effect of altering the level of farnesyltransferase activity in those cells.

Overexpression of the proteins of the instant invention may be accomplished by first constructing a chimeric gene in which the coding region is operably linked to a promoter capable of directing expression of a gene in the desired tissues at the desired stage of development. For reasons of convenience, the chimeric gene may comprise promoter sequences and translation leader sequences derived from the same genes. 3' Non-coding sequences encoding transcription termination signals may also be provided. The instant chimeric gene may also comprise one or more introns in order to facilitate gene expression.

Plasmid vectors comprising the instant chimeric gene can then be constructed. The choice of plasmid vector is dependent upon the method that will be used to transform host plants. The skilled artisan is well aware of the genetic elements that must be present on the plasmid vector in order to successfully transform, select and propagate host cells containing the chimeric gene. The skilled artisan will also recognize that different independent transformation events will result in different levels and patterns of expression (Jones et al. (1985) *EMBO J. 4*:2411-2418; De Almeida et al. (1989) *Mol. Gen. Genetics 218*:78-86), and thus that multiple events must be screened in order to obtain lines displaying the desired expression level and pattern. Such screening may be accomplished by Southern analysis of DNA, Northern analysis of mRNA expression, Western analysis of protein expression, or phenotypic analysis.

For some applications it may be useful to direct the instant polypeptides to different cellular compartments, or to facilitate its secretion from the cell. It is thus envisioned that the chimeric gene described above may be further supplemented by altering the coding sequence to encode the instant polypeptides with appropriate intracellular targeting sequences such as transit sequences (Keegstra (1989) *Cell 56*:247-253), signal sequences or sequences encoding endoplasmic reticulum localization (Chrispeels (1991) *Ann. Rev. Plant Phys. Plant Mol. Biol. 42*:21-53), or nuclear localization signals (Raikhel (1992) *Plant*

Phys.100:1627-1632) added and/or with targeting sequences that are already present removed. While the references cited give examples of each of these, the list is not exhaustive and more targeting signals of utility may be discovered in the future.

It may also be desirable to reduce or eliminate expression of genes encoding the instant polypeptides in plants for some applications. In order to accomplish this, a chimeric gene designed for co-suppression of the instant polypeptide can be constructed by linking a gene or gene fragment encoding that polypeptide to plant promoter sequences. Alternatively, a chimeric gene designed to express antisense RNA for all or part of the instant nucleic acid fragment can be constructed by linking the gene or gene fragment in reverse orientation to plant promoter sequences. Either the co-suppression or antisense chimeric genes could be introduced into plants via transformation wherein expression of the corresponding endogenous genes are reduced or eliminated.

Molecular genetic solutions to the generation of plants with altered gene expression have a decided advantage over more traditional plant breeding approaches. Changes in plant phenotypes can be produced by specifically inhibiting expression of one or more genes by antisense inhibition or cosuppression (U.S. Patent Nos. 5,190,931, 5,107,065 and 5,283,323). An antisense or cosuppression construct would act as a dominant negative regulator of gene activity. While conventional mutations can yield negative regulation of gene activity these effects are most likely recessive. The dominant negative regulation available with a transgenic approach may be advantageous from a breeding perspective. In addition, the ability to restrict the expression of specific phenotype to the reproductive tissues of the plant by the use of tissue specific promoters may confer agronomic advantages relative to conventional mutations which may have an effect in all tissues in which a mutant gene is ordinarily expressed.

The person skilled in the art will know that special considerations are associated with the use of antisense or cosuppression technologies in order to reduce expression of particular genes. For example, the proper level of expression of sense or antisense genes may require the use of different chimeric genes utilizing different regulatory elements known to the skilled artisan. Once transgenic plants are obtained by one of the methods described above, it will be necessary to screen individual transgenics for those that most effectively display the desired phenotype. Accordingly, the skilled artisan will develop methods for screening large numbers of transformants. The nature of these screens will generally be chosen on practical grounds, and is not an inherent part of the invention. For example, one can screen by looking for changes in gene expression by using antibodies specific for the protein encoded by the gene being suppressed, or one could establish assays that specifically measure enzyme activity. A preferred method will be one which allows large numbers of samples to be processed rapidly, since it will be expected that a large number of transformants will be negative for the desired phenotype.

The instant polypeptides (or portions thereof) may be produced in heterologous host cells, particularly in the cells of microbial hosts, and can be used to prepare antibodies to the these proteins by methods well known to those skilled in the art. The antibodies are useful for detecting the polypeptides of the instant invention *in situ* in cells or *in vitro* in cell extracts. Preferred heterologous host cells for production of the instant polypeptides are microbial hosts. Microbial expression systems and expression vectors containing regulatory sequences that direct high level expression of foreign proteins are well known to those skilled in the art. Any of these could be used to construct a chimeric gene for production of the instant polypeptides. This chimeric gene could then be introduced into appropriate microorganisms via transformation to provide high level expression of the encoded farnesyltransferase subunit. An example of a vector for high level expression of the instant polypeptides in a bacterial host is provided (Example 7).

All or a substantial portion of the nucleic acid fragments of the instant invention may also be used as probes for genetically and physically mapping the genes that they are a part of, and as markers for traits linked to those genes. Such information may be useful in plant breeding in order to develop lines with desired phenotypes. For example, the instant nucleic acid fragments may be used as restriction fragment length polymorphism (RFLP) markers. Southern blots (Maniatis) of restriction-digested plant genomic DNA may be probed with the nucleic acid fragments of the instant invention. The resulting banding patterns may then be subjected to genetic analyses using computer programs such as MapMaker (Lander et al. (1987) *Genomics 1*:174-181) in order to construct a genetic map. In addition, the nucleic acid fragments of the instant invention may be used to probe Southern blots containing restriction endonuclease-treated genomic DNAs of a set of individuals representing parent and progeny of a defined genetic cross. Segregation of the DNA polymorphisms is noted and used to calculate the position of the instant nucleic acid sequence in the genetic map previously obtained using this population (Botstein et al. (1980) *Am. J. Hum. Genet. 32*:314-331).

The production and use of plant gene-derived probes for use in genetic mapping is described in Bernatzky and Tanksley (1986) *Plant Mol. Biol. Reporter 4*:37-41. Numerous publications describe genetic mapping of specific cDNA clones using the methodology outlined above or variations thereof. For example, F2 intercross populations, backcross populations, randomly mated populations, near isogenic lines, and other sets of individuals may be used for mapping. Such methodologies are well known to those skilled in the art.

Nucleic acid probes derived from the instant nucleic acid sequences may also be used for physical mapping (i.e., placement of sequences on physical maps; *see* Hoheisel et al. In: *Nonmammalian Genomic Analysis: A Practical Guide*, Academic press 1996, pp. 319-346, and references cited therein).

In another embodiment, nucleic acid probes derived from the instant nucleic acid sequences may be used in direct fluorescence *in situ* hybridization (FISH) mapping (Trask (1991) *Trends Genet.* 7:149-154). Although current methods of FISH mapping favor use of large clones (several to several hundred KB; see Laan et al. (1995) *Genome Res.* 5:13-20), improvements in sensitivity may allow performance of FISH mapping using shorter probes.

A variety of nucleic acid amplification-based methods of genetic and physical mapping may be carried out using the instant nucleic acid sequences. Examples include allele-specific amplification (Kazazian (1989) *J. Lab. Clin. Med.* 11:95-96), polymorphism of PCR-amplified fragments (CAPS; Sheffield et al. (1993) *Genomics* 16:325-332), allele-specific ligation (Landegren et al. (1988) *Science* 241:1077-1080), nucleotide extension reactions (Sokolov (1990) *Nucleic Acid Res.* 18:3671), Radiation Hybrid Mapping (Walter et al. (1997) *Nat. Genet.* 7:22-28) and Happy Mapping (Dear and Cook (1989) *Nucleic Acid Res.* 17:6795-6807). For these methods, the sequence of a nucleic acid fragment is used to design and produce primer pairs for use in the amplification reaction or in primer extension reactions. The design of such primers is well known to those skilled in the art. In methods employing PCR-based genetic mapping, it may be necessary to identify DNA sequence differences between the parents of the mapping cross in the region corresponding to the instant nucleic acid sequence. This, however, is generally not necessary for mapping methods.

Loss of function mutant phenotypes may be identified for the instant cDNA clones either by targeted gene disruption protocols or by identifying specific mutants for these genes contained in a maize population carrying mutations in all possible genes (Ballinger and Benzer (1989) *Proc. Natl. Acad. Sci USA* 86:9402-9406; Koes et al. (1995) *Proc. Natl. Acad. Sci USA* 92:8149-8153; Bensen et al. (1995) *Plant Cell* 7:75-84). The latter approach may be accomplished in two ways. First, short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols in conjunction with a mutation tag sequence primer on DNAs prepared from a population of plants in which Mutator transposons or some other mutation-causing DNA element has been introduced (see Bensen, *supra*). The amplification of a specific DNA fragment with these primers indicates the insertion of the mutation tag element in or near the plant gene encoding the instant polypeptides. Alternatively, the instant nucleic acid fragment may be used as a hybridization probe against PCR amplification products generated from the mutation population using the mutation tag sequence primer in conjunction with an arbitrary genomic site primer, such as that for a restriction enzyme site-anchored synthetic adaptor. With either method, a plant containing a mutation in the endogenous gene encoding the instant polypeptides can be identified and obtained. This mutant plant can then be used to determine or confirm the natural function of the instant polypeptides disclosed herein.

EXAMPLES

The present invention is further defined in the following Examples, in which all parts and percentages are by weight and degrees are Celsius, unless otherwise stated. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

EXAMPLE 1

Composition of cDNA Libraries; Isolation and Sequencing of cDNA Clones

cDNA libraries representing mRNAs from various corn, rice, soybean and wheat tissues were prepared. The characteristics of the libraries are described below.

TABLE 2

cDNA Libraries from Corn, Rice, Soybean and Wheat

Library	Tissue	Clone
cen3n	Corn endosperm 20 days after pollination*	cen3n.pk0052.a2
	Corn nucellus tissue, 5 days after silking*	p0127.cntbu18r
rlr24	Rice leaf 15 days after germination, 24 hours after infection of strain *Magnaporthe grisea* 4360-R-62 (AVR2-YAMO); Resistant	rlr24.pk0007.d6
rls6	Rice leaf 15 days after germination, 6 hours after infection of *Magnaporthe grisea* strain 4360-R-67 (AVR2-YAMO); susceptible	rls6.pk0017.g2
		sfl1.pk0086.h10
sgs2c	Soybean seeds 14 hours after germination	sgs2c.pk006.n4
sgs2c	Soybean seeds 14 hours after germination	sgs2c.pk002.g2
src1c	Soybean 8 day old root infected with cyst nematode *Heterodera glycinesis*	src1c.pk002.f15
wdk2c	Wheat developing kernel, 7 days after anthesis	wdk2c.pk013.d24
wr1	Wheat root from 7 day old seedling	wr1.pk0110.b1

*These libraries were normalized essentially as described in U.S. Patent No. 5,482,845, incorporated herein by reference.

cDNA libraries may be prepared by any one of many methods available. For example, the cDNAs may be introduced into plasmid vectors by first preparing the cDNA libraries in Uni-ZAP™ XR vectors according to the manufacturer's protocol (Stratagene Cloning Systems, La Jolla, CA). The Uni-ZAP™ XR libraries are converted into plasmid libraries according to the protocol provided by Stratagene. Upon conversion, cDNA inserts will be contained in the plasmid vector pBluescript. In addition, the cDNAs may be

introduced directly into precut Bluescript II SK(+) vectors (Stratagene) using T4 DNA ligase (New England Biolabs), followed by transfection into DH10B cells according to the manufacturer's protocol (GIBCO BRL Products). Once the cDNA inserts are in plasmid vectors, plasmid DNAs are prepared from randomly picked bacterial colonies containing recombinant pBluescript plasmids, or the insert cDNA sequences are amplified via polymerase chain reaction using primers specific for vector sequences flanking the inserted cDNA sequences. Amplified insert DNAs or plasmid DNAs are sequenced in dye-primer sequencing reactions to generate partial cDNA sequences (expressed sequence tags or "ESTs"; see Adams et al., (1991) *Science 252*:1651-1656). The resulting ESTs are analyzed using a Perkin Elmer Model 377 fluorescent sequencer.

EXAMPLE 2

Identification of cDNA Clones

cDNA clones encoding farnesyltransferase subunits were identified by conducting BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol. 215*:403-410; see also www.ncbi.nlm.nih.gov/BLAST/) searches for similarity to sequences contained in the BLAST "nr" database (comprising all non-redundant GenBank CDS translations, sequences derived from the 3-dimensional structure Brookhaven Protein Data Bank, the last major release of the SWISS-PROT protein sequence database, EMBL, and DDBJ databases). The cDNA sequences obtained in Example 1 were analyzed for similarity to all publicly available DNA sequences contained in the "nr" database using the BLASTN algorithm provided by the National Center for Biotechnology Information (NCBI). The DNA sequences were translated in all reading frames and compared for similarity to all publicly available protein sequences contained in the "nr" database using the BLASTX algorithm (Gish and States (1993) *Nat. Genet. 3*:266-272) provided by the NCBI. For convenience, the P-value (probability) of observing a match of a cDNA sequence to a sequence contained in the searched databases merely by chance as calculated by BLAST are reported herein as "pLog" values, which represent the negative of the logarithm of the reported P-value. Accordingly, the greater the pLog value, the greater the likelihood that the cDNA sequence and the BLAST "hit" represent homologous proteins.

EXAMPLE 3

Characterization of cDNA Clones Encoding Farnesyltransferase Alpha Subunits

The BLASTX search using the EST sequences from clones listed in Table 3 revealed similarity of the polypeptides encoded by the cDNAs to farnesyltransferase alpha subunit from *Pisum sativum* (NCBI Identifier No. gi 2246442) and *Arabidopsis thaliana* (NCBI Identifier No. gi 3142698). Shown in Table 3 are the BLAST results for individual ESTs ("EST"), the sequences of the entire cDNA inserts comprising the indicated cDNA clones ("FIS"), or contigs assembled from two or more ESTs ("Contig"):

TABLE 3

BLAST Results for Sequences Encoding Polypeptides Homologous to *Pisum sativum* and *Arabidopsis thaliana* Farnesyltransferase Alpha Subunits

Clone	Status	BLAST pLog Score
cen3n.pk0052.a2	FIS	115.00 (gi 2246442)
rls6.pk0017.g2	FIS	121.00 (gi 3142698)
src1c.pk002.f15	FIS	152.00 (gi 2246442)
sgs2c.pk006.n4	FIS	150.00 (gi 2246442)
Contig composed of: wdk2c.pk013.d24 wr1.pk0110.b1	Contig	114.00 (gi 3142698)

The data in Table 4 represents a calculation of the percent identity of the amino acid sequences set forth in SEQ ID NOs:2, 4, 6, 8 and 10 and the *Pisum sativum* and *Arabidopsis thaliana* sequences (SEQ ID NO:19 and 20 respectively). The percent identity between the amino acid sequences set forth in SEQ ID NOs:2, 4, 6, 8 and 10 ranged between 55-99%.

TABLE 4

Percent Identity of Amino Acid Sequences Deduced From the Nucleotide Sequences of cDNA Clones Encoding Polypeptides Homologous to *Pisum sativum* and *Arabidopsis thaliana* Farnesyltransferase Alpha Subunits

SEQ ID NO.	Percent Identity to
2	60%
4	62%
6	77%
8	76%
10	62%

Sequence alignments and percent identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS.* 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. Sequence alignments and BLAST scores and probabilities indicate that the nucleic acid fragments comprising the instant cDNA clones encode a substantial portion of a farnesyltransferase alpha subunit. These sequences represent the first corn, rice, soybean and wheat sequences encoding a farnesyltransferase alpha subunit.

EXAMPLE 4

Characterization of cDNA Clones Encoding Farnesyltransferase Beta Subunits

The BLASTX search using the EST sequences from clones listed in Table 5 revealed similarity of the polypeptides encoded by the cDNAs to farnesyltransferase beta subunit from *Lycopersicon esculentum* (NCBI Identifier No. gi 1815668), *Pisum sativum* (NCBI Identifier No. gi 417482) and *Pisum sativum* (NCBI Identifier No. gi 169049). Shown in Table 5 are the BLAST results for individual ESTs ("EST"), the sequences of the entire cDNA inserts comprising the indicated cDNA clones ("FIS"), or contigs assembled from two or more ESTs ("Contig"):

TABLE 5

BLAST Results for Sequences Encoding Polypeptides Homologous to *Lycopersicon esculentum* and *Pisum sativum* Farnesyltransferase Beta Subunits

Clone	Status	BLAST pLog Score
p0127.cntbu18r	FIS	149.00 (gi 1815668)
rlr24.pk0007.d6	FIS	110.00 (gi 1815668)
sfl1.pk0086.h10	FIS	>254.00 (gi 417482)
sgs2c.pk002.g2	EST	62.00 (gi 169049)

The data in Table 6 represents a calculation of the percent identity of the amino acid sequences set forth in SEQ ID NOs:12, 14, 16 and 18 and the *Lycopersicon esculentum* (gi 1815668), *Pisum sativum* (gi 417482) and *Pisum sativum* (gi 169049) sequences. The percent identity between the amino acid sequences set forth in SEQ ID NOs:12, 14, 16 and 18 ranged between 50-81%.

TABLE 6

Percent Identity of Amino Acid Sequences Deduced From the Nucleotide Sequences of cDNA Clones Encoding Polypeptides Homologous to *Lycopersicon esculentum* and *Pisum sativum* Farnesyltransferase Beta Subunits

SEQ ID NO.	Percent Identity to
p0127.cntbu18r	55%
rlr24.pk0007.d6	57%
sfl1.pk0086.h10	77%
sgs2c.pk002.g2	62%

Sequence alignments and percent identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS. 5*:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for

pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. Sequence alignments and BLAST scores and probabilities indicate that the nucleic acid fragments comprising the instant cDNA clones encode a substantial portion of a farnesyltransferase beta subunit. These sequences represent the first corn, rice, soybean and wheat sequences encoding a farnesyltransferase beta subunit.

EXAMPLE 5

Expression of Chimeric Genes in Monocot Cells

A chimeric gene comprising a cDNA encoding the instant polypeptides in sense orientation with respect to the maize 27 kD zein promoter that is located 5' to the cDNA fragment, and the 10 kD zein 3' end that is located 3' to the cDNA fragment, can be constructed. The cDNA fragment of this gene may be generated by polymerase chain reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites (NcoI or SmaI) can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the digested vector pML103 as described below. Amplification is then performed in a standard PCR. The amplified DNA is then digested with restriction enzymes NcoI and SmaI and fractionated on an agarose gel. The appropriate band can be isolated from the gel and combined with a 4.9 kb NcoI-SmaI fragment of the plasmid pML103. Plasmid pML103 has been deposited under the terms of the Budapest Treaty at ATCC (American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209), and bears accession number ATCC 97366. The DNA segment from pML103 contains a 1.05 kb SalI-NcoI promoter fragment of the maize 27 kD zein gene and a 0.96 kb SmaI-SalI fragment from the 3' end of the maize 10 kD zein gene in the vector pGem9Zf(+) (Promega). Vector and insert DNA can be ligated at 15°C overnight, essentially as described (Maniatis). The ligated DNA may then be used to transform *E. coli* XL1-Blue (Epicurian Coli XL-1 Blue™; Stratagene). Bacterial transformants can be screened by restriction enzyme digestion of plasmid DNA and limited nucleotide sequence analysis using the dideoxy chain termination method (Sequenase™ DNA Sequencing Kit; U.S. Biochemical). The resulting plasmid construct would comprise a chimeric gene encoding, in the 5' to 3' direction, the maize 27 kD zein promoter, a cDNA fragment encoding the instant polypeptides, and the 10 kD zein 3' region.

The chimeric gene described above can then be introduced into corn cells by the following procedure. Immature corn embryos can be dissected from developing caryopses derived from crosses of the inbred corn lines H99 and LH132. The embryos are isolated 10 to 11 days after pollination when they are 1.0 to 1.5 mm long. The embryos are then placed with the axis-side facing down and in contact with agarose-solidified N6 medium (Chu et al. (1975) *Sci. Sin. Peking* 18:659-668). The embryos are kept in the dark at 27°C. Friable embryogenic callus consisting of undifferentiated masses of cells with somatic

proembryoids and embryoids borne on suspensor structures proliferates from the scutellum of these immature embryos. The embryogenic callus isolated from the primary explant can be cultured on N6 medium and sub-cultured on this medium every 2 to 3 weeks.

The plasmid, p35S/Ac (obtained from Dr. Peter Eckes, Hoechst Ag, Frankfurt, Germany) may be used in transformation experiments in order to provide for a selectable marker. This plasmid contains the *Pat* gene (see European Patent Publication 0 242 236) which encodes phosphinothricin acetyl transferase (PAT). The enzyme PAT confers resistance to herbicidal glutamine synthetase inhibitors such as phosphinothricin. The *pat* gene in p35S/Ac is under the control of the 35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) *Nature* 313:810-812) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*.

The particle bombardment method (Klein et al. (1987) *Nature* 327:70-73) may be used to transfer genes to the callus culture cells. According to this method, gold particles (1 μm in diameter) are coated with DNA using the following technique. Ten μg of plasmid DNAs are added to 50 μL of a suspension of gold particles (60 mg per mL). Calcium chloride (50 μL of a 2.5 M solution) and spermidine free base (20 μL of a 1.0 M solution) are added to the particles. The suspension is vortexed during the addition of these solutions. After 10 minutes, the tubes are briefly centrifuged (5 sec at 15,000 rpm) and the supernatant removed. The particles are resuspended in 200 μL of absolute ethanol, centrifuged again and the supernatant removed. The ethanol rinse is performed again and the particles resuspended in a final volume of 30 μL of ethanol. An aliquot (5 μL) of the DNA-coated gold particles can be placed in the center of a Kapton™ flying disc (Bio-Rad Labs). The particles are then accelerated into the corn tissue with a Biolistic™ PDS-1000/He (Bio-Rad Instruments, Hercules CA), using a helium pressure of 1000 psi, a gap distance of 0.5 cm and a flying distance of 1.0 cm.

For bombardment, the embryogenic tissue is placed on filter paper over agarose-solidified N6 medium. The tissue is arranged as a thin lawn and covered a circular area of about 5 cm in diameter. The petri dish containing the tissue can be placed in the chamber of the PDS-1000/He approximately 8 cm from the stopping screen. The air in the chamber is then evacuated to a vacuum of 28 inches of Hg. The macrocarrier is accelerated with a helium shock wave using a rupture membrane that bursts when the He pressure in the shock tube reaches 1000 psi.

Seven days after bombardment the tissue can be transferred to N6 medium that contains gluphosinate (2 mg per liter) and lacks casein or proline. The tissue continues to grow slowly on this medium. After an additional 2 weeks the tissue can be transferred to fresh N6 medium containing gluphosinate. After 6 weeks, areas of about 1 cm in diameter of actively growing callus can be identified on some of the plates containing the glufosinate-

supplemented medium. These calli may continue to grow when sub-cultured on the selective medium.

Plants can be regenerated from the transgenic callus by first transferring clusters of tissue to N6 medium supplemented with 0.2 mg per liter of 2,4-D. After two weeks the tissue can be transferred to regeneration medium (Fromm et al. (1990) *Bio/Technology 8*:833-839).

EXAMPLE 6

Expression of Chimeric Genes in Dicot Cells

A seed-specific expression cassette composed of the promoter and transcription terminator from the gene encoding the β subunit of the seed storage protein phaseolin from the bean *Phaseolus vulgaris* (Doyle et al. (1986) *J. Biol. Chem. 261*:9228-9238) can be used for expression of the instant polypeptides in transformed soybean. The phaseolin cassette includes about 500 nucleotides upstream (5') from the translation initiation codon and about 1650 nucleotides downstream (3') from the translation stop codon of phaseolin. Between the 5' and 3' regions are the unique restriction endonuclease sites Nco I (which includes the ATG translation initiation codon), SmaI, KpnI and XbaI. The entire cassette is flanked by Hind III sites.

The cDNA fragment of this gene may be generated by polymerase chain reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the expression vector. Amplification is then performed as described above, and the isolated fragment is inserted into a pUC18 vector carrying the seed expression cassette.

Soybean embroys may then be transformed with the expression vector comprising sequences encoding the instant polypeptides. To induce somatic embryos, cotyledons, 3-5 mm in length dissected from surface sterilized, immature seeds of the soybean cultivar A2872, can be cultured in the light or dark at 26°C on an appropriate agar medium for 6-10 weeks. Somatic embryos which produce secondary embryos are then excised and placed into a suitable liquid medium. After repeated selection for clusters of somatic embryos which multiplied as early, globular staged embryos, the suspensions are maintained as described below.

Soybean embryogenic suspension cultures can maintained in 35 mL liquid media on a rotary shaker, 150 rpm, at 26°C with florescent lights on a 16:8 hour day/night schedule. Cultures are subcultured every two weeks by inoculating approximately 35 mg of tissue into 35 mL of liquid medium.

Soybean embryogenic suspension cultures may then be transformed by the method of particle gun bombardment (Klein et al. (1987) *Nature* (London) *327*:70-73, U.S. Patent

No. 4,945,050). A DuPont Biolistic™ PDS1000/HE instrument (helium retrofit) can be used for these transformations.

A selectable marker gene which can be used to facilitate soybean transformation is a chimeric gene composed of the 35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) *Nature 313*:810-812), the hygromycin phosphotransferase gene from plasmid pJR225 (from *E. coli*; Gritz et al.(1983) *Gene 25*:179-188) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*. The seed expression cassette comprising the phaseolin 5' region, the fragment encoding the instant polypeptides and the phaseolin 3' region can be isolated as a restriction fragment. This fragment can then be inserted into a unique restriction site of the vector carrying the marker gene.

To 50 μL of a 60 mg/mL 1 μm gold particle suspension is added (in order): 5 μL DNA (1 μg/μL), 20 μl spermidine (0.1 M), and 50 μL $CaCl_2$ (2.5 M). The particle preparation is then agitated for three minutes, spun in a microfuge for 10 seconds and the supernatant removed. The DNA-coated particles are then washed once in 400 μL 70% ethanol and resuspended in 40 μL of anhydrous ethanol. The DNA/particle suspension can be sonicated three times for one second each. Five μL of the DNA-coated gold particles are then loaded on each macro carrier disk.

Approximately 300-400 mg of a two-week-old suspension culture is placed in an empty 60x15 mm petri dish and the residual liquid removed from the tissue with a pipette. For each transformation experiment, approximately 5-10 plates of tissue are normally bombarded. Membrane rupture pressure is set at 1100 psi and the chamber is evacuated to a vacuum of 28 inches mercury. The tissue is placed approximately 3.5 inches away from the retaining screen and bombarded three times. Following bombardment, the tissue can be divided in half and placed back into liquid and cultured as described above.

Five to seven days post bombardment, the liquid media may be exchanged with fresh media, and eleven to twelve days post bombardment with fresh media containing 50 mg/mL hygromycin. This selective media can be refreshed weekly. Seven to eight weeks post bombardment, green, transformed tissue may be observed growing from untransformed, necrotic embryogenic clusters. Isolated green tissue is removed and inoculated into individual flasks to generate new, clonally propagated, transformed embryogenic suspension cultures. Each new line may be treated as an independent transformation event. These suspensions can then be subcultured and maintained as clusters of immature embryos or regenerated into whole plants by maturation and germination of individual somatic embryos.

EXAMPLE 7

Expression of Chimeric Genes in Microbial Cells

The cDNAs encoding the instant polypeptides can be inserted into the T7 *E. coli* expression vector pBT430. This vector is a derivative of pET-3a (Rosenberg et al. (1987) *Gene 56*:125-135) which employs the bacteriophage T7 RNA polymerase/T7 promoter

system. Plasmid pBT430 was constructed by first destroying the EcoR I and Hind III sites in pET-3a at their original positions. An oligonucleotide adaptor containing EcoR I and Hind III sites was inserted at the BamH I site of pET-3a. This created pET-3aM with additional unique cloning sites for insertion of genes into the expression vector. Then, the Nde I site at the position of translation initiation was converted to an Nco I site using oligonucleotide-directed mutagenesis. The DNA sequence of pET-3aM in this region, 5'-CATATGG, was converted to 5'-CCCATGG in pBT430.

Plasmid DNA containing a cDNA may be appropriately digested to release a nucleic acid fragment encoding the protein. This fragment may then be purified on a 1% NuSieve GTG™ low melting agarose gel (FMC). Buffer and agarose contain 10 μg/ml ethidium bromide for visualization of the DNA fragment. The fragment can then be purified from the agarose gel by digestion with GELase™ (Epicentre Technologies) according to the manufacturer's instructions, ethanol precipitated, dried and resuspended in 20 μL of water. Appropriate oligonucleotide adapters may be ligated to the fragment using T4 DNA ligase (New England Biolabs, Beverly, MA). The fragment containing the ligated adapters can be purified from the excess adapters using low melting agarose as described above. The vector pBT430 is digested, dephosphorylated with alkaline phosphatase (NEB) and deproteinized with phenol/chloroform as described above. The prepared vector pBT430 and fragment can then be ligated at 16°C for 15 hours followed by transformation into DH5 electrocompetent cells (GIBCO BRL). Transformants can be selected on agar plates containing LB media and 100 μg/mL ampicillin. Transformants containing the gene encoding the instant polypeptides are then screened for the correct orientation with respect to the T7 promoter by restriction enzyme analysis.

For high level expression, a plasmid clone with the cDNA insert in the correct orientation relative to the T7 promoter can be transformed into *E. coli* strain BL21(DE3) (Studier et al. (1986) *J. Mol. Biol. 189*:113-130). Cultures are grown in LB medium containing ampicillin (100 mg/L) at 25°C. At an optical density at 600 nm of approximately 1, IPTG (isopropylthio-β-galactoside, the inducer) can be added to a final concentration of 0.4 mM and incubation can be continued for 3 h at 25°. Cells are then harvested by centrifugation and re-suspended in 50 μL of 50 mM Tris-HCl at pH 8.0 containing 0.1 mM DTT and 0.2 mM phenyl methylsulfonyl fluoride. A small amount of 1 mm glass beads can be added and the mixture sonicated 3 times for about 5 seconds each time with a microprobe sonicator. The mixture is centrifuged and the protein concentration of the supernatant determined. One μg of protein from the soluble fraction of the culture can be separated by SDS-polyacrylamide gel electrophoresis. Gels can be observed for protein bands migrating at the expected molecular weight.

CLAIMS

What is claimed is:

1. An isolated polynucleotide comprising a nucleotide sequence encoding a first polypeptide of at least 300 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of a corn farnesyltransferase polypeptide of SEQ ID NO:2, a rice farnesyltransferase polypeptide of SEQ ID NO:4, a soybean farnesyltransferase polypeptide of SEQ ID NO:6, a soybean farnesyltransferase polypeptide of SEQ ID NO:8, a wheat farnesyltransferase polypeptide of SEQ ID NO:10, a corn farnesyltransferase polypeptide of SEQ ID NO:12, a rice farnesyltransferase polypeptide of SEQ ID NO:14, a soybean farnesyltransferase polypeptide of SEQ ID NO:16, and a soybean farnesyltransferase polypeptide of SEQ ID NO:18.

2. An isolated polynucleotide comprising the complement of the polynucleotide of Claim 1.

3. The isolated polynucleotide of Claim 1, wherein the nucleotide sequence comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15 and 17 that codes for the polypeptide selected from the group consisting of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16 and 18.

4. The isolated polynucleotide of Claim 1 which is DNA.

5. The isolated polynucleotide of Claim 1 which is RNA.

6. A chimeric gene comprising the isolated polynucleotide of Claim 1 or Claim 2 operably linked to suitable regulatory sequences.

7. An isolated host cell comprising the chimeric gene of Claim 6.

8. An isolated host cell comprising an isolated polynucleotide of Claim 1.

9. The isolated host cell of Claim 8, wherein the host cell is yeast.

10. The isolated host cell of Claim 8, wherein the host cell is a bacterial cell.

11. The isolated host cell of Claim 8, wherein the host cell is a plant cell.

12. A virus comprising the isolated polynucleotide of Claim 1.

13. A process for producing an isolated host cell comprising the chimeric gene of Claim 6, the process comprising either transforming or transfecting an isolated compatible host cell with the chimeric gene of Claim 6.

14. A farnesyltransferase polypeptide of at least 300 amino acids that has at least 80% homology based on the Clustal method of alignment compared to a polypeptide selected from the group consisting of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16 and 18.

15. A method of selecting an isolated polynucleotide that affects the level of expression of a farnesyltransferase polypeptide in a plant cell, the method comprising the steps of:

constructing an isolated polynucleotide comprising a nucleotide sequence of at least one of 30 contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17 and the complement of such nucleotide sequences;

introducing the isolated polynucleotide into a plant cell;

measuring the level of farnesyltransferase polypeptide in the plant cell containing the polynucleotide; and

comparing the level of farnesyltransferase polypeptide in the plant cell containing the isolated polynucleotide with the level of farnesyltransferase polypeptide in a plant cell that does not contain the polynucleotide.

16. The method of Claim 15 wherein the isolated polynucleotide comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, and 17 that codes for the polypeptide selected from the group consisting of SEQ ID NO:2, 4, 6, 8,10, 12, 14, 16 and 18.

17. The method of Claim 15 wherein the isolated polynucleotide is DNA.

18. The method of Claim 15 wherein the isolated polynucleotide is RNA.

19. The method of Claim 15 wherein the isolated polynucleotide is a chimeric gene comprising the nucleotide sequence operably linked to suitable regulatory sequences.

20. A method of selecting an isolated polynucleotide that affects the level of expression of farnesyltransferase polypeptide in a plant cell, the method comprising the steps of:

constructing the isolated polynucleotide of Claim 1;

introducing the isolated polynucleotide into a plant cell;

measuring the level of farnesyltransferase polypeptide in the plant cell containing the polynucleotide; and

comparing the level of farnesyltransferase polypeptide in the plant cell containing the isolated polynucleotide with the level of farnesyltransferase polypeptide in a plant cell that does not contain the isolated polynucleotide.

21. A method of obtaining a nucleic acid fragment encoding a substantial portion of a farnesyltransferase gene comprising the steps of:

synthesizing an oligonucleotide primer comprising a nucleotide sequence of at least one of 40 contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17 and the complement of such nucleotide sequences; and

amplifying a nucleic acid sequence using the oligonucleotide primer.

22. A method of obtaining a nucleic acid fragment encoding all or a substantial portion of the amino acid sequence encoding a farnesyltransferase protein comprising the steps of:

probing a cDNA or genomic library with an isolated polynucleotide comprising a nucleotide sequence of at least one of 30 contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, and the complement of such nucleotide sequences;

identifying a DNA clone that hybridizes with the isolated polynucleotide;

isolating the identified DNA clone; and

sequencing the cDNA or genomic fragment that comprises the isolated DNA clone.

23. The isolated polynucleotide of Claim 1, wherein the first polypeptide is compared to the corn farnesyltransferase polypeptide of SEQ ID NO:2.

24. The isolated polynucleotide of Claim 1, wherein the first polypeptide is compared to the rice farnesyltransferase polypeptide of SEQ ID NO:4.

25. The isolated polynucleotide of Claim 1, wherein the first polypeptide is compared to the soybean farnesyltransferase polypeptide of SEQ ID NO:6.

26. The isolated polynucleotide of Claim 1, wherein the first polypeptide is compared to the soybean farnesyltransferase polypeptide of SEQ ID NO:8.

27. The isolated polynucleotide of Claim 1, wherein the first polypeptide is compared to the wheat farnesyltransferase polypeptide of SEQ ID NO:10.

28. The isolated polynucleotide of Claim 1, wherein the first polypeptide is compared to the corn farnesyltransferase polypeptide of SEQ ID NO:12.

29. The isolated polynucleotide of Claim 1, wherein the first polypeptide is compared to the rice farnesyltransferase polypeptide of SEQ ID NO:14.

30. The isolated polynucleotide of Claim 1, wherein the first polypeptide is compared to the soybean farnesyltransferase polypeptide of SEQ ID NO:16.

31. The isolated polynucleotide of Claim 1, wherein the first polypeptide is compared to the soybean farnesyltransferase polypeptide of SEQ ID NO:18.

SEQUENCE LISTING

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Powell, Wayne

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atttggcgca agagcagact tcctctatca gcttagtaac caaagtaatt aaagggcaac 1140
tctgtgttat gtgtaaccta gtttattgaa actggatgtt tatttattat tatttttat 1200
gttgtcatgt atctgtttgt gcaaatttat ctttttgtca tgccattact ggcatttgag 1260
tgtaaggatt gaaagccatg cagaataaga aatttaagtt tttttttccg ttgaaaaaaa 1320
aaaaaaaaaa aaa                                                    1333
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<210> 8
<211> 358
<212> PRT
<213> Glycine max

<400> 8

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Met Glu Ser Gly Ser Ser Glu Gly Glu Glu Val Gln Gln Arg Val Pro
1               5                  10                  15
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Leu Arg Glu Arg Val Glu Trp Ser Asp Val Thr Pro Val Pro Gln Asn
20                  25                  30

Asp Gly Pro Asn Pro Val Val Pro Ile Gln Tyr Thr Glu Glu Phe Ser
35                  40                  45

Glu Val Met Asp Tyr Phe Arg Ala Val Tyr Leu Thr Asp Glu Arg Ser
50                  55                  60

Pro Arg Ala Leu Ala Leu Thr Ala Glu Ala Val Gln Phe Asn Ser Gly
65                  70                  75                  80

Asn Tyr Thr Val Trp His Phe Arg Arg Leu Leu Leu Glu Ser Leu Lys
85                  90                  95

Val Asp Leu Asn Asp Glu Leu Glu Phe Val Glu Arg Met Ala Ala Gly
100                 105                 110

Asn Ser Lys Asn Tyr Gln Met Trp Cys Asp Ala Leu Leu Cys Ser Phe
115                 120                 125

Phe His Thr Leu His His Arg Arg Trp Val Ala Glu Lys Leu Gly Pro
130                 135                 140

Glu Ala Arg Asn Asn Glu Leu Glu Phe Thr Lys Lys Ile Leu Ser Val
145                 150                 155                 160

Asp Ala Lys His Tyr His Ala Trp Ser His Arg Gln Trp Ala Leu Gln
165                 170                 175

Thr Leu Gly Gly Trp Glu Asp Glu Leu Asn Tyr Cys Thr Glu Leu Leu
180                 185                 190

Lys Glu Asp Ile Phe Asn Asn Ser Ala Trp Asn Gln Arg Tyr Phe Val
195                 200                 205

Ile Thr Arg Ser Pro Phe Leu Gly Gly Leu Lys Ala Met Arg Glu Ser
210                 215                 220

Glu Val Leu Tyr Thr Ile Glu Ala Ile Ile Ala Tyr Pro Glu Asn Glu
225                 230                 235                 240

Ser Ser Trp Arg Tyr Leu Arg Gly Leu Tyr Lys Gly Glu Thr Thr Ser
245                 250                 255

Trp Val Asn Asp Pro Gln Val Ser Ser Val Cys Leu Lys Ile Leu Arg
260                 265                 270

Thr Lys Ser Asn Tyr Val Phe Ala Leu Ser Thr Ile Leu Asp Leu Ile
275                 280                 285

Cys Phe Gly Tyr Gln Pro Asn Glu Asp Ile Arg Asp Ala Ile Asp Ala
290                 295                 300

Leu Lys Thr Ala Asp Met Asp Lys Gln Asp Leu Asp Asp Asp Glu Lys
305                 310                 315                 320

Gly Glu Gln Gln Asn Leu Asn Ile Ala Arg Asn Ile Cys Ser Ile Leu
325                 330                 335
```

Lys Gln Val Asp Pro Ile Arg Thr Asn Tyr Trp Ile Trp Arg Lys Ser
340 345 350

Arg Leu Pro Leu Ser Ala
355

<210> 9
<211> 1339
<212> DNA
<213> Triticum aestivum

<400> 9
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gcggcgactt ccgcgaggtc atggactact tccgcgccct ctacgccgcc ggcgagcgca 120
gcccccgcgc cctccgcctc accgccgacg ccatccacct caaccccggc aactacactg 180
tatggcattt caggcgcgtt gttctagagg cactggatgc tgatttattg ctagaaatgc 240
attttgtgga ccaaattgct gaatctaatc caaaaaatta ccaagtctgg catcacaaga 300
gatggcttgc tgagaaaata ggaccagatg ctgcaaatag tgaacatgac ttcacaagga 360
agatacttgc tatggatgct aaaaactacc atgcttggtc ccataggcag tgggttcttc 420
aagcattggg tggatgggag agtgaactgc agtactgcaa ccagcttctt gaggaagatg 480
tcttcaataa ctcagcttgg aatcagagat accttgtggt aacacgatca ccaattcttg 540
ggggccttgc ggcaatgcgc gactcagaag tagattacac agttgaggcc attatggtga 600
accctcagaa tgaaagcccc tggagatacc tcagaggttt atataaggat gataacaatt 660
tgctggtggc tgataatcgc atttctgatg cttgcctcaa ggtcctgaat aaggattgga 720
catgcgtatt tgctttgagc ttcctgcttg atcttcttcg catgggtttg cagccttcga 780
atgaacttaa aggaaccatc gaagcaatgg agaactctga tcctgaaacg ggacatgctg 840
atattgcagt agctgtctgc tcaatcctgc agaaatgtga tcccctgcgg ataaactact 900
ggtcatggta ccagaccact ctttcttctt agacatctga aaattcagct gaagacagtt 960
ttagcagcat gatgtaaact caatcgaagg ggttgacgca gtgtatgaaa aacctttcct 1020
gtgatcttgg tgcggagcaa tttgtactga ttttactggg aaaaatcaat caatgacagc 1080
atgcccaaca atgtcttgtg tgaatatgtt actgcctgat attcacatgt tagcagaatg 1140
agaataacca atcaaactcc aacgagcaga ttgttacagt aacggccact ggtggtgtga 1200
aaatcctgaa atctgcttca gtcactttgc cttgtttaca gttgagtctg ttgttgtgat 1260
ctgtacctaa tgcatgtaca caatcatcaa attattagtt tttgtaccaa tgagtattcg 1320
atgaaaaaaa aaaaaaaaa 1339

<210> 10
<211> 309
<212> PRT
<213> Triticum aestivum

<400> 10
Asp Val Ala Pro Leu Pro Gln Ala Asp Gly Pro Cys Pro Val Val Ser
1 5 10 15

Ile Ala Tyr Arg Gly Asp Phe Arg Glu Val Met Asp Tyr Phe Arg Ala
20 25 30

Leu Tyr Ala Ala Gly Glu Arg Ser Pro Arg Ala Leu Arg Leu Thr Ala
35 40 45

Asp Ala Ile His Leu Asn Pro Gly Asn Tyr Thr Val Trp His Phe Arg
50 55 60

Arg Val Val Leu Glu Ala Leu Asp Ala Asp Leu Leu Leu Glu Met His
65 70 75 80

Phe Val Asp Gln Ile Ala Glu Ser Asn Pro Lys Asn Tyr Gln Val Trp
85 90 95

```
His His Lys Arg Trp Leu Ala Glu Lys Ile Gly Pro Asp Ala Ala Asn
100                 105                 110

Ser Glu His Asp Phe Thr Arg Lys Ile Leu Ala Met Asp Ala Lys Asn
115                 120                 125

Tyr His Ala Trp Ser His Arg Gln Trp Val Leu Gln Ala Leu Gly Gly
130                 135                 140

Trp Glu Ser Glu Leu Gln Tyr Cys Asn Gln Leu Leu Glu Glu Asp Val
145                 150                 155                 160

Phe Asn Asn Ser Ala Trp Asn Gln Arg Tyr Leu Val Val Thr Arg Ser
165                 170                 175

Pro Ile Leu Gly Gly Leu Ala Ala Met Arg Asp Ser Glu Val Asp Tyr
180                 185                 190

Thr Val Glu Ala Ile Met Val Asn Pro Gln Asn Glu Ser Pro Trp Arg
195                 200                 205

Tyr Leu Arg Gly Leu Tyr Lys Asp Asp Asn Asn Leu Leu Val Ala Asp
210                 215                 220

Asn Arg Ile Ser Asp Ala Cys Leu Lys Val Leu Asn Lys Asp Trp Thr
225                 230                 235                 240

Cys Val Phe Ala Leu Ser Phe Leu Leu Asp Leu Leu Arg Met Gly Leu
245                 250                 255

Gln Pro Ser Asn Glu Leu Lys Gly Thr Ile Glu Ala Met Glu Asn Ser
260                 265                 270

Asp Pro Glu Thr Gly His Ala Asp Ile Ala Val Ala Val Cys Ser Ile
275                 280                 285

Leu Gln Lys Cys Asp Pro Leu Arg Ile Asn Tyr Trp Ser Trp Tyr Gln
290                 295                 300

Thr Thr Leu Ser Ser
305
```

<210> 11
<211> 1359
<212> DNA
<213> Zea mays

<400> 11

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atggacccct ccccgcagtc gacgccgccc accggagacg acccggcagc ggcggcggat   60
cccgacctac cgaggctcac ggtgacgcag gtggagcaga tgaaggtgga ggccagggtt  120
ggcgacatct accgctccct cttcggggcc gcgcccaaca cgaaatccat catgctagag  180
ctgtggcgtg atcagcatat cgagtatctg acgcctgggc tgaggcatat gggaccagcc  240
tttcatgttc tagatgccaa tcgcccttgg ctatgctact ggatggttca tccacttgct  300
ttgctggatg aagcacttga tgatgatctt gagaatgata tcatagactt cttagctcga  360
tgtcaggata aagatggtgg atatagtggt ggacctggac agttgcctca cctagctacg  420
acttatgctg ctgtaaatac acttgtgaca atagggagcg aaagagcatt gtcatcaatc  480
aataggggca acctgtacaa ttttatgctg cagatgaaag atgtatcagg tgctttcaga  540
atgcatgatg gtggcgaaat tgatgtccgt gcttcctaca ccgctatatc ggttgccagc  600
cttgtgaata ttcttgattt taaactggca aaaggtgtag gcgactacat agcaagatgt  660
caaacttatg aaggtggtat tgctggggag ccttatgctg aagcacatgg tgggtataca  720
```

```
ttctgtggat tggctgcttt gatcctgctt aatgaggcag agaaagttga cttgcctagt  780
ttgattggct gggtggcttt tcgtcaagga gtggaatgcg gatttcaagg acgaactaat  840
aaattggttg atggttgcta ctccttttgg cagggagctg ccattgcttt cacacaaaag  900
ttaattacga ttgttgataa gcaattgaag tcctcgtatt cctgcaaaag gccatcagga  960
gaggatgcct gcagcaccag ttcatatggg tgcaccgcga aaaagtcttc ctctgctgtg 1020
gactatgcga agtttggatt tgattttata caacagagca accaaattgg cccactcttc 1080
cataacattg ccctgcaaca atacatccta ctttgttctc aggtactaga gggaggcttg 1140
agggataagc ctggaaagaa cagagatcac tatcattcat gctactgcct cagtggcctc 1200
gcagttagcc agtacagtgc catgactgat actggttcgt gcccattacc tcagcatgtg 1260
cttggaccgt actctaattt gctggagcca atccatccac tctacaatgt tgtcctagat 1320
aagtaccata cagcctatga gttcttctca gaagagtga                         1359
```

<210> 12
<211> 452
<212> PRT
<213> Zea mays

<400> 12

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Met Asp Pro Ser Pro Gln Ser Thr Pro Pro Thr Gly Asp Asp Pro Ala
1               5                  10                  15

Ala Ala Ala Asp Pro Asp Leu Pro Arg Leu Thr Val Thr Gln Val Glu
20                  25                  30

Gln Met Lys Val Glu Ala Arg Val Gly Asp Ile Tyr Arg Ser Leu Phe
35                  40                  45

Gly Ala Ala Pro Asn Thr Lys Ser Ile Met Leu Glu Leu Trp Arg Asp
50                  55                  60

Gln His Ile Glu Tyr Leu Thr Pro Gly Leu Arg His Met Gly Pro Ala
65                  70                  75                  80

Phe His Val Leu Asp Ala Asn Arg Pro Trp Leu Cys Tyr Trp Met Val
85                  90                  95

His Pro Leu Ala Leu Leu Asp Glu Ala Leu Asp Asp Asp Leu Glu Asn
100                 105                 110

Asp Ile Ile Asp Phe Leu Ala Arg Cys Gln Asp Lys Asp Gly Gly Tyr
115                 120                 125

Ser Gly Gly Pro Gly Gln Leu Pro His Leu Ala Thr Thr Tyr Ala Ala
130                 135                 140

Val Asn Thr Leu Val Thr Ile Gly Ser Glu Arg Ala Leu Ser Ser Ile
145                 150                 155                 160

Asn Arg Gly Asn Leu Tyr Asn Phe Met Leu Gln Met Lys Asp Val Ser
165                 170                 175

Gly Ala Phe Arg Met His Asp Gly Gly Glu Ile Asp Val Arg Ala Ser
180                 185                 190

Tyr Thr Ala Ile Ser Val Ala Ser Leu Val Asn Ile Leu Asp Phe Lys
195                 200                 205

Leu Ala Lys Gly Val Gly Asp Tyr Ile Ala Arg Cys Gln Thr Tyr Glu
210                 215                 220
```

Gly Gly Ile Ala Gly Glu Pro Tyr Ala Glu Ala His Gly Gly Tyr Thr
225 230 235 240

Phe Cys Gly Leu Ala Ala Leu Ile Leu Leu Asn Glu Ala Glu Lys Val
245 250 255

Asp Leu Pro Ser Leu Ile Gly Trp Val Ala Phe Arg Gln Gly Val Glu
260 265 270

Cys Gly Phe Gln Gly Arg Thr Asn Lys Leu Val Asp Gly Cys Tyr Ser
275 280 285

Phe Trp Gln Gly Ala Ala Ile Ala Phe Thr Gln Lys Leu Ile Thr Ile
290 295 300

Val Asp Lys Gln Leu Lys Ser Ser Tyr Ser Cys Lys Arg Pro Ser Gly
305 310 315 320

Glu Asp Ala Cys Ser Thr Ser Ser Tyr Gly Cys Thr Ala Lys Lys Ser
325 330 335

Ser Ser Ala Val Asp Tyr Ala Lys Phe Gly Phe Asp Phe Ile Gln Gln
340 345 350

Ser Asn Gln Ile Gly Pro Leu Phe His Asn Ile Ala Leu Gln Gln Tyr
355 360 365

Ile Leu Leu Cys Ser Gln Val Leu Glu Gly Gly Leu Arg Asp Lys Pro
370 375 380

Gly Lys Asn Arg Asp His Tyr His Ser Cys Tyr Cys Leu Ser Gly Leu
385 390 395 400

Ala Val Ser Gln Tyr Ser Ala Met Thr Asp Thr Gly Ser Cys Pro Leu
405 410 415

Pro Gln His Val Leu Gly Pro Tyr Ser Asn Leu Leu Glu Pro Ile His
420 425 430

Pro Leu Tyr Asn Val Val Leu Asp Lys Tyr His Thr Ala Tyr Glu Phe
435 440 445

Phe Ser Glu Glu
450

<210> 13
<211> 1031
<212> DNA
<213> Oryza sativa

<400> 13
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ccgccttccg cgccgccgac caccgcgccc atggaccccc cctcgccgcc gccgccgccg 120
ccatatcctc ctgctgctgc tgagggcggt ccggcagcgg atagccaggc cgctgagctg 180
ccccggctga ctgtgacgca ggtggagcag atgaaggtgg aggcgaaggt gggcgaaatc 240
taccgcgtcc tcttcggcaa cgcgcccaac gccaattccc tcatgttaga gctgtggcgt 300
gagcagcatg ttgagtattt gacgagaggg ctgaaacatc ttggaccaag cttccatgtg 360
ctcgatgcca atcgaccttg gctgtgctac tggattattc atgcacttgc tctgttggat 420
gaaatacctg acgatgttga ggatgatatt gtggacttct tatctcgatg tcaggacaaa 480
gatggtggtt atggcggagg acctggacag ttgcctcatc tcgctacaac ttatgctgct 540

gtaaatacac ttgtaactat agggagtgaa agggcactat catcggtaaa cagggacaac 600
ctgtacaagt tcatgcttcg gatgaaagat acatcgggag ctttcagaat gcatgatggt 660
ggtgaaatag atgttcgtgc tagctatact gcaatatcgg ttgccagcct tgtgaacatt 720
cttgatggtg aactagcaaa aggtgttgga aattacataa caaggtgtca aacctatgaa 780
ggtggcattg ctggggaacc gtatgctgaa gctcatggtg ggtacacttt ttgtgggctg 840
gctacgatga tcctgcttaa cgaagtggac aaacttgatt tggctagctt gattggctgg 900
gtggcatttc gccaaggagt ggaatgtgga tttcaaggac gaactaataa attggttgat 960
ggttgctact ccttttggca gggagctgct cttgctttaa ctgttcaccg cgtggcgccg 1020
actgccaaac g 1031

<210> 14
<211> 313
<212> PRT
<213> Oryza sativa

<400> 14
Met Asp Pro Pro Ser Pro Pro Pro Pro Pro Pro Tyr Pro Pro Ala Ala
1 5 10 15

Ala Glu Gly Gly Pro Ala Ala Asp Ser Gln Ala Ala Glu Leu Pro Arg
20 25 30

Leu Thr Val Thr Gln Val Glu Gln Met Lys Val Glu Ala Lys Val Gly
35 40 45

Glu Ile Tyr Arg Val Leu Phe Gly Asn Ala Pro Asn Ala Asn Ser Leu
50 55 60

Met Leu Glu Leu Trp Arg Glu Gln His Val Glu Tyr Leu Thr Arg Gly
65 70 75 80

Leu Lys His Leu Gly Pro Ser Phe His Val Leu Asp Ala Asn Arg Pro
85 90 95

Trp Leu Cys Tyr Trp Ile Ile His Ala Leu Ala Leu Leu Asp Glu Ile
100 105 110

Pro Asp Asp Val Glu Asp Asp Ile Val Asp Phe Leu Ser Arg Cys Gln
115 120 125

Asp Lys Asp Gly Gly Tyr Gly Gly Gly Pro Gly Gln Leu Pro His Leu
130 135 140

Ala Thr Thr Tyr Ala Ala Val Asn Thr Leu Val Thr Ile Gly Ser Glu
145 150 155 160

Arg Ala Leu Ser Ser Val Asn Arg Asp Asn Leu Tyr Lys Phe Met Leu
165 170 175

Arg Met Lys Asp Thr Ser Gly Ala Phe Arg Met His Asp Gly Gly Glu
180 185 190

Ile Asp Val Arg Ala Ser Tyr Thr Ala Ile Ser Val Ala Ser Leu Val
195 200 205

Asn Ile Leu Asp Gly Glu Leu Ala Lys Gly Val Gly Asn Tyr Ile Thr
210 215 220

Arg Cys Gln Thr Tyr Glu Gly Gly Ile Ala Gly Glu Pro Tyr Ala Glu
225 230 235 240

```
Ala His Gly Gly Tyr Thr Phe Cys Gly Leu Ala Thr Met Ile Leu Leu
245                 250                 255

Asn Glu Val Asp Lys Leu Asp Leu Ala Ser Leu Ile Gly Trp Val Ala
260                 265                 270

Phe Arg Gln Gly Val Glu Cys Gly Phe Gln Gly Arg Thr Asn Lys Leu
275                 280                 285

Val Asp Gly Cys Tyr Ser Phe Trp Gln Gly Ala Ala Leu Ala Leu Thr
290                 295                 300

Val His Arg Val Ala Pro Thr Ala Lys
305                 310
```

<210> 15
<211> 1504
<212> DNA
<213> Glycine max

<400> 15

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gcacgaggac aaatccgccg ccgccgccgc cgtgtccgac ggtgagtcaa cgtgagcaat   60
ggatggtaga gtcgcaggtg tttcagattt accaactctt cgccaccatt cctcgcaacg  120
cccaaaccct catgttggag cttcaacgcg ataatcacat gcagtatgtc tccaaaggcc  180
ttcgccatct cagttccgca ttttccgttt tggacgctaa tcgaccctgg ctctgctact  240
ggatcttcca ctccattgct ttgtcgggag aatccgtcga tgatgaactc gaagataacg  300
ctatcgattt tcttaaccgt tgccaggatc cgaatggtgg atatgccggg ggaccaggcc  360
agatgcctca tattgccaca acttatgctg ctgttaattc acttattact ttgggtggtg  420
agaaatccct ggcatcaatt aatagagata aactgtatgg gtttctgcgg cggatgaagc  480
aaccaaatgg tggattcagg atgcatgatg aaggtgaaat tgatgttcga gcttgctaca  540
ctgccatttc tgttgcaagt gttttgaaca ttttggatga tgagctgatc cagaatgttg  600
gagactacat tataagctgt caaacatatg agggtggcat tgctggtgag cctggttctg  660
aggctcatgg tgggtacacc ttttgtggat tagctacaat gattctgatt ggtgaggtta  720
atcacttgga tctgcctcga ttagttgact gggtggtatt ccgacaaggt aaggaatgtg  780
gattccaggg gagaacaaat aaactggtgg atggatgcta ttccttttgg cagggaggtg  840
ctgttgctct attgcaaaga ttatcttcta ttatcaacaa acagatggaa gagacatcac  900
agatttttgc ggtatcttat gtatctgaag caaaagaaag tttggatgga acctctagtc  960
atgcaacatg ccgtggtgag catgaaggca ccagtgaatc cagttcatct gattttaaaa 1020
atattgccta taaatttatt aatgagtgga gagcacaaga accacttttt cacagtattg 1080
ctttacagca atatattctc ttatgtgcac aggagcaaga gggtggactg agagacaaac 1140
cgggtaaacg tagagatcat tatcacacat gttactgttt aagtggactc tcattgtgcc 1200
agtatagttg gtcaaagcac ccagattctc caccactgcc taatctagta ttaggcccct 1260
actctaatct cttagaacca atccaccccc tctttaatgt tgtcttggga cgatatcgtg 1320
aagctcatga attcttcttt actgagtcgt gaccactggt tttagctacc aacaacttta 1380
tttgtataat gtaaaataaa ttcattggaa catataaatg taaaacagca ttggattaaa 1440
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 1500
aaaa                                                               1504
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<210> 16
<211> 429
<212> PRT
<213> Glycine max

<400> 16

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Met Val Glu Ser Gln Val Phe Gln Ile Tyr Gln Leu Phe Ala Thr Ile
1               5                  10                  15

Pro Arg Asn Ala Gln Thr Leu Met Leu Glu Leu Gln Arg Asp Asn His
20                  25                  30
```

```
Met Gln Tyr Val Ser Lys Gly Leu Arg His Leu Ser Ser Ala Phe Ser
35                  40                  45

Val Leu Asp Ala Asn Arg Pro Trp Leu Cys Tyr Trp Ile Phe His Ser
50                  55                  60

Ile Ala Leu Ser Gly Glu Ser Val Asp Asp Glu Leu Glu Asp Asn Ala
65                  70                  75                  80

Ile Asp Phe Leu Asn Arg Cys Gln Asp Pro Asn Gly Gly Tyr Ala Gly
85                  90                  95

Gly Pro Gly Gln Met Pro His Ile Ala Thr Thr Tyr Ala Ala Val Asn
100                 105                 110

Ser Leu Ile Thr Leu Gly Gly Glu Lys Ser Leu Ala Ser Ile Asn Arg
115                 120                 125

Asp Lys Leu Tyr Gly Phe Leu Arg Arg Met Lys Gln Pro Asn Gly Gly
130                 135                 140

Phe Arg Met His Asp Glu Gly Glu Ile Asp Val Arg Ala Cys Tyr Thr
145                 150                 155                 160

Ala Ile Ser Val Ala Ser Val Leu Asn Ile Leu Asp Asp Glu Leu Ile
165                 170                 175

Gln Asn Val Gly Asp Tyr Ile Ile Ser Cys Gln Thr Tyr Glu Gly Gly
180                 185                 190

Ile Ala Gly Glu Pro Gly Ser Glu Ala His Gly Gly Tyr Thr Phe Cys
195                 200                 205

Gly Leu Ala Thr Met Ile Leu Ile Gly Glu Val Asn His Leu Asp Leu
210                 215                 220

Pro Arg Leu Val Asp Trp Val Val Phe Arg Gln Gly Lys Glu Cys Gly
225                 230                 235                 240

Phe Gln Gly Arg Thr Asn Lys Leu Val Asp Gly Cys Tyr Ser Phe Trp
245                 250                 255

Gln Gly Gly Ala Val Ala Leu Leu Gln Arg Leu Ser Ser Ile Ile Asn
260                 265                 270

Lys Gln Met Glu Glu Thr Ser Gln Ile Phe Ala Val Ser Tyr Val Ser
275                 280                 285

Glu Ala Lys Glu Ser Leu Asp Gly Thr Ser Ser His Ala Thr Cys Arg
290                 295                 300

Gly Glu His Glu Gly Thr Ser Glu Ser Ser Ser Ser Asp Phe Lys Asn
305                 310                 315                 320

Ile Ala Tyr Lys Phe Ile Asn Glu Trp Arg Ala Gln Glu Pro Leu Phe
325                 330                 335

His Ser Ile Ala Leu Gln Gln Tyr Ile Leu Leu Cys Ala Gln Glu Gln
340                 345                 350
```

Glu Gly Gly Leu Arg Asp Lys Pro Gly Lys Arg Arg Asp His Tyr His
355 360 365

Thr Cys Tyr Cys Leu Ser Gly Leu Ser Leu Cys Gln Tyr Ser Trp Ser
370 375 380

Lys His Pro Asp Ser Pro Pro Leu Pro Asn Leu Val Leu Gly Pro Tyr
385 390 395 400

Ser Asn Leu Leu Glu Pro Ile His Pro Leu Phe Asn Val Val Leu Gly
405 410 415

Arg Tyr Arg Glu Ala His Glu Phe Phe Phe Thr Glu Ser
420 425

<210> 17
<211> 533
<212> DNA
<213> Glycine max

<220>
<221> unsure
<222> (499)

<220>
<221> unsure
<222> (525)

<400> 17
gagagagata cgaatccggc ggcggcgcca ccgtgtccga cggtgagtca acgggaccag 60
tggatggtag agtcgcaggt gtttcagatt taccaactct ttgccaccat tcctggcagc 120
gcccaaaacc tcatgttaga gctgcaacgc gataatcaca tgcagtatct ctccaaaggc 180
ctacgccatc tcagttccgc gttttctgtc ttggacgcta atcgaccctg gctctgttac 240
tggatcttcc attccattgc tttgctggga gaatccgtcg acgacgaact cgaagataac 300
actatcgatt ttcttaaccg ttgccaggat ccgaatggtg gatatgctgg gggaccaggc 360
cagatgcctc acattgccac aacatatgct gcagttaata cacttattac tttgggtggt 420
cagaaatcct ggcatcaatt aataggtgag ataaactgta tgggtttctg cggcggatga 480
agcaatcaaa tggggggant caagatgcat gatgaaagga gaaanttgat gtc 533

<210> 18
<211> 141
<212> PRT
<213> Glycine max

<400> 18
Asp Thr Asn Pro Ala Ala Ala Pro Pro Cys Pro Thr Val Ser Gln Arg
1 5 10 15

Asp Gln Trp Met Val Glu Ser Gln Val Phe Gln Ile Tyr Gln Leu Phe
20 25 30

Ala Thr Ile Pro Gly Ser Ala Gln Asn Leu Met Leu Glu Leu Gln Arg
35 40 45

Asp Asn His Met Gln Tyr Leu Ser Lys Gly Leu Arg His Leu Ser Ser
50 55 60

Ala Phe Ser Val Leu Asp Ala Asn Arg Pro Trp Leu Cys Tyr Trp Ile
65 70 75 80

Phe His Ser Ile Ala Leu Leu Gly Glu Ser Val Asp Asp Glu Leu Glu
85 90 95

Asp Asn Thr Ile Asp Phe Leu Asn Arg Cys Gln Asp Pro Asn Gly Gly
100 105 110

Tyr Ala Gly Gly Pro Gly Gln Met Pro His Ile Ala Thr Thr Tyr Ala
115 120 125

Ala Val Asn Thr Leu Ile Thr Leu Gly Gly Gln Lys Ser
130 135 140

<210> 19
<211> 333
<212> PRT
<213> Pisum sativum

<400> 19
Met Ala Gly Asn Ile Glu Val Glu Glu Asp Asp Arg Val Pro Leu Arg
1 5 10 15

Leu Arg Pro Glu Trp Ser Asp Val Thr Pro Ile Pro Gln Asp Asp Gly
20 25 30

Pro Ser Pro Val Val Pro Ile Asn Tyr Ser Glu Glu Phe Ser Glu Val
35 40 45

Met Asp Tyr Phe Arg Ala Val Tyr Phe Ala Lys Glu Leu Ser Ser Arg
50 55 60

Ala Leu Ala Leu Thr Ala Glu Ala Ile Gly Leu Asn Ala Gly Asn Tyr
65 70 75 80

Thr Val Trp His Phe Arg Arg Leu Leu Leu Glu Ser Leu Lys Val Asp
85 90 95

Leu His Val Glu Arg Glu Phe Val Glu Arg Val Ala Ser Gly Asn Ser
100 105 110

Lys Asn Tyr Gln Ile Trp His His Arg Arg Trp Val Ala Glu Lys Leu
115 120 125

Gly Pro Glu Ala Arg Asn Ser Glu Leu Glu Phe Thr Lys Lys Ile Leu
130 135 140

Ser Val Asp Ala Lys His Tyr His Ala Trp Ser His Arg Gln Trp Val
145 150 155 160

Leu Gln Asn Leu Gly Gly Trp Glu Asp Glu Leu Ser Tyr Cys Ser Glu
165 170 175

Leu Leu Ala Glu Asp Ile Phe Asn Asn Ser Ala Trp Asn Gln Arg Tyr
180 185 190

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225 230 235 240

Thr Leu Tyr Val Asn Asp Ala Gln Val Ser Ser Leu Cys Leu Lys Ile
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Leu Lys Thr Lys Ser Asn Tyr Leu Phe Ala Leu Ser Thr Leu Leu Asp
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35 40 45

Ile Tyr Phe Ser Asp Glu Arg Ser Pro Arg Ala Leu Arg Leu Thr Glu
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Glu Thr Leu Leu Leu Asn Ser Gly Asn Tyr Thr Val Trp His Phe Arg
65 70 75 80

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100 105 110

His His Arg Arg Trp Val Ala Glu Lys Leu Gly Pro Asp Val Ala Gly
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Arg Glu Leu Glu Phe Thr Arg Arg Val Leu Ser Leu Asp Ala Lys His
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Tyr His Ala Trp Ser His Arg Gln Trp Thr Leu Arg Ala Leu Gly Gly
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Trp Glu Asp Glu Leu Asp Tyr Cys His Glu Leu Leu Glu Ala Asp Val
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Phe Asn Asn Ser Ala Trp Asn Gln Arg Tyr Tyr Val Ile Thr Gln Ser
180                 185                 190

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275                 280                 285

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Ser Val Ala Asn Ile Leu Asn Ile Val Asp Asp Glu Leu Ile His Gly
180                 185                 190

Val Gly Asn Tyr Ile Leu Ser Cys Gln Thr Tyr Glu Gly Gly Ile Ala
195                 200                 205

Gly Glu Pro Gly Ser Glu Ala His Gly Gly Tyr Thr Phe Cys Gly Leu
210                 215                 220

Ala Ala Met Ile Leu Ile Asn Glu Val Asp Arg Leu Asp Leu Pro Gly
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His Val Gln Asp Thr Phe Pro Leu Gly Gln Ala Gly Ala Cys Gln Glu
325                 330                 335

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340                 345                 350

Asn Arg Pro Ile Ala Met Arg Pro Leu Phe Asp Ser Met Tyr Leu Gln
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Gln Tyr Val Leu Leu Cys Ser Gln Ile Glu Val Gly Gly Phe Arg Asp
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Lys Pro Gly Lys Gly Arg Asp Tyr Tyr His Thr Cys Tyr Cys Leu Ser
385                 390                 395                 400

Gly Leu Ser Ile Ala Gln Tyr Ser Trp Thr Asp Glu Ala Asp Ser Thr
405                 410                 415

Pro Leu Pro Arg Asp Val Phe Gly Pro Tyr Ser Lys Cys Leu Leu Glu
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Gln Val His Pro Leu Phe Asn Val Val Leu Asp Arg Tyr Tyr Glu Ala
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Cys Tyr Trp Ile Ile His Ser Ile Ala Leu Leu Gly Glu Ser Ile Asp
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Pro Asn Gly Gly Tyr Ala Gly Gly Pro Gly Gln Met Pro His Leu Ala
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Cys Gln Thr Tyr Glu Gly Gly Leu Ala Gly Glu Pro Gly Ser Glu Ala
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290                 295                 300

Cys Ser Ser Asp Val Lys Asn Ile Gly Tyr Asn Phe Ile Ser Glu Trp
305                 310                 315                 320

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Lys Arg Arg Asp His Tyr His Ser Cys Tyr Cys Leu Ser Gly Leu Ser
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370                 375                 380

Lys Val Val Met Gly Pro Tyr Ser Asn Leu Leu Glu Pro Ile His Pro
385                 390                 395                 400

Leu Phe Asn Val Val Leu Asp Arg Tyr Arg Glu Ala His Glu Phe Phe
405                 410                 415

Ser Gln Leu
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<400> 23

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Phe Ala Asn Ile Pro Pro Asn Ala Gln Ser Ile Ile Arg Pro Trp Leu
35                  40                  45

Cys Tyr Trp Ile Ile His Ser Ile Ala Leu Leu Gly Glu Ser Ile Asp
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Asp Asp Leu Glu Asp Asn Thr Val Asp Phe Leu Asn Arg Cys Gln Asp
65                  70                  75                  80

Pro Asn Gly Gly Tyr Ala Gly Gly Pro Gly Gln Met Pro His Leu Ala
85                  90                  95

Thr Thr Tyr Ala Ala Val Asn Thr Leu Ile Thr Leu Gly Gly Glu Lys
100                 105                 110
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Asp Val Arg Ala Cys Tyr Thr Ala Ile Ser Val Ala Ser Val Leu Asn
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Ile Leu Asp Asp Glu Leu Ile Lys Asn Val Gly Asp Phe Ile Leu Ser
165                 170                 175

Cys Gln Thr Tyr Glu Gly Gly Leu Ala Gly Glu Pro Gly Ser Glu Ala
180                 185                 190

His Gly Gly Tyr Thr Phe Cys Gly Leu Ala Ala Met Ile Leu Ile Gly
195                 200                 205

Glu Val Asn Arg Leu Asp Leu Pro Arg Leu Leu Asp Trp Val Val Phe
210                 215                 220

Arg Gln Gly Lys Glu Cys Gly Phe Gln Gly Arg Thr Asn Lys Leu Val
225                 230                 235                 240

Asp Gly Cys Tyr Ser Phe Trp Gln Gly Gly Ala Val Ala Leu Leu Gln
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Arg Leu His Ser Ile Ile Asp Glu Gln Met Ala Glu Ala Ser Gln Phe
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Val Thr Val Ser Asp Ala Pro Glu Glu Lys Glu Cys Leu Asp Gly Thr
275                 280                 285

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340                 345                 350

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355                 360                 365

Leu Cys Gln Tyr Ser Trp Ser Lys Arg Pro Asp Ser Pro Pro Leu Pro
370                 375                 380

Lys Val Val Met Gly Pro Tyr Ser Asn Leu Leu Glu Pro Ile His Pro
385                 390                 395                 400

Leu Phe Asn Val Val Leu Asp Arg Tyr Arg Glu Ala His Glu Phe Phe
405                 410                 415

Ser Gln Leu
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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(72) Inventors; and

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(81) Designated States: AE, AL, AU, BA, BB, BG, BR, CA, CN, CR, CU, CZ, EE, GD, GE, HR, HU, ID, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, SL, TR, TT, UA, US, UZ, VN, YU, ZA, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
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17 August 2000 (17.08.00)

(54) Title: PLANT FARNESYLTRANSFERASES

(57) Abstract

This invention relates to an isolated nucleic acid fragment encoding a farnesyltransferase subunit. The invention also relates to the construction of a chimeric gene encoding all or a portion of the farnesyltransferase subunit, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of the farnesyltransferase subunit in a transformed host cell.

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EE	Estonia	LR	Liberia	SG	Singapore		

INTERNATIONAL SEARCH REPORT

Int. .ional Application No
PCT/US 99/20419

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N9/10 C12N15/29 C12N15/54 C12N15/70 C12N15/80

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	QIAN ET AL: "Protein farnesyltransferase in plants: molecular characterization and involvement in cell cycle control" PLANT CELL, vol. 8, 1996, page 2381-2394 XP002090873 ROCKVILLE US ISSN: 1040-4651 page 2390 -page 2392	1,2,4, 6-8,10, 11,13,14
A		22, 24-26,29

-/--

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

* Special categories of cited documents :

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"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 April 2000	15. 5. 00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	De Kok, A

INTERNATIONAL SEARCH REPORT

Int. ional Application No
PCT/US 99/20419

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	YANG Z ET AL: "PROTEIN FARNESYLTRANSFERASE IN PLANTS 1 MOLECULAR CLONING AND EXPRESSION OF A HOMOLOG OF THE BETA SUBUNIT FROM THE GARDEN PEA" PLANT PHYSIOLOGY, vol. 101, no. 2, 1993, page 667-674 XP000647522 ROCKVILLE US ISSN: 0032-0889 cited in the application abstract; figure 2	1-5,22, 30
A	---	22,31
A	CUTLER S. ET AL: "A protein farnesyl transferase involved in abscisic acid signal transduction in arabidopsis" SCIENCE, vol. 273, August 1996 (1996-08), page 1239-1241 XP002090871 WASHINGTON US ISSN: 0036-8075	1
A	--- YALOVSKY S. ET AL.: "Plant farnesyl transferase can restore yeast ras signaling and mating" MOLECULAR AND CELLULAR BIOLOGY.,. vol. 17, no. 4, 1997, pages 1986-1994, XP000864327 WASHINGTON., US ISSN: 0270-7306 abstract	1-5,22, 27,29
A	--- HATA S. ET AL.: "cDNA cloning of squalene synthase genes from mono- and dicotyledonous plants, and expression of the gene in rice." PLANT AND CELL PHYSIOLOGY, vol. 38, no. 12, 1997, pages 1409-1413, XP000864329 abstract	1
A	--- MERLOT ET AL: "Genetic analysis of abscisic acid signal transduction" PLANT PHYSIOLOGY, vol. 114, 1997, page 751-757 XP002090872 ROCKVILLE US the whole document	1
A	--- SKOCZYLAS E. ET AL.: "Protein farnesyltransferase in plants" BIOCHIMIE, vol. 78, no. 2, 1996, pages 139-143, XP000864561 page 141, paragraph 2; figure 2	1-5,14, 22,27

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/20419

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1, 2
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1, 2

Claims 1 and 2 refer to a polynucleotide or the complement thereof, which encodes a polypeptide with a defined sequence.
Back-translation of the polypeptide generates a very large number of nucleic acid sequences. It is not possible to search for this enormous set of sequences. Therefore, the search has been restricted to the conventional nucleic acid/nucleic acid, protein/protein and protein/six-frame translated nucleic acid comparisons.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-22 partly and 23, 28 completely

An isolated polynucleotide comprising a polynucleotide encoding a polypeptide of at least 300 amino acids, said polypeptide having at least 80 % identity with a corn farnesyltransferase polypeptide of SEQ.ID.NO: 2 or SEQ.ID.NO: 12; a chimeric gene comprising said polynucleotide; a host cell comprising said polynucleotide; a virus comprising said polynucleotide; a farnesyltransferase polypeptide of at least 300 amino acids having at least 80% homology with a corn farnesyltransferase polypeptide of SEQ.ID.NO: 2 or SEQ.ID.NO: 12; a method of selecting an isolated polynucleotide that affects the level of a farnesyltransferase in a plant cell and a method of obtaining a nucleic acid fragment encoding a substantial portion of a corn farnesyltransferase gene comprising using a primer derived from SEQ.ID.NO: 1 or SEQ.ID.NO: 11.

2. Claims: 1-22 partly and 24, 29 completely

An isolated polynucleotide comprising a polynucleotide encoding a polypeptide of at least 300 amino acids, said polypeptide having at least 80 % identity with a rice farnesyltransferase polypeptide of SEQ.ID.NO: 4 or SEQ.ID.NO: 14; a chimeric gene comprising said polynucleotide; a host cell comprising said polynucleotide; a virus comprising said polynucleotide; a farnesyltransferase polypeptide of at least 300 amino acids having at least 80% homology with a rice farnesyltransferase polypeptide of SEQ.ID.NO: 4 or SEQ.ID.NO: 14; a method of selecting an isolated polynucleotide that affects the level of a farnesyltransferase in a plant cell and a method of obtaining a nucleic acid fragment encoding a substantial portion of a rice farnesyltransferase gene comprising using a primer derived from SEQ.ID.NO: 3 or SEQ.ID.NO: 13.

3. Claims: 1-22 partly and 25, 26, 30, 31 completely

An isolated polynucleotide comprising a polynucleotide encoding a polypeptide of at least 300 amino acids, said polypeptide having at least 80 % identity with a soybean farnesyltransferase polypeptide of SEQ.ID.NO: 6,8,16 or 18; a chimeric gene comprising said polynucleotide; a host cell comprising said polynucleotide; a virus comprising said polynucleotide; a farnesyltransferase polypeptide of at least 300 amino acids having at least 80% homology with a soybean farnesyltransferase polypeptide of SEQ.ID.NO: 6,8,16 or 18; a method of selecting an isolated polynucleotide that affects the level of a farnesyltransferase in a plant cell and a method of obtaining a nucleic acid fragment encoding a

substantial portion of a soybean farnesyltransferase gene comprising using a primer derived from SEQ.ID.NO: 5,7,15 or 17.

4. Claims: 1-22 partly and 27 completely

An isolated polynucleotide comprising a polynucleotide encoding a polypeptide of at least 300 amino acids, said polypeptide having at least 80 % identity with a wheat farnesyltransferase polypeptide of SEQ.ID.NO: 10; a chimeric gene comprising said polynucleotide; a host cell comprising said polynucleotide; a virus comprising said polynucleotide; a farnesyltransferase polypeptide of at least 300 amino acids having at least 80% homology with a wheat farnesyltransferase polypeptide of SEQ.ID.NO: 10; a method of selecting an isolated polynucleotide that affects the level of a farnesyltransferase in a plant cell and a method of obtaining a nucleic acid fragment encoding a substantial portion of a wheat farnesyltransferase gene comprising using a primer derived from SEQ.ID.NO: 9.